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(71)(72) Applicant and Inventor: BARENKAMP, Stephen, J. [US/US]; 16 Villawood Lane, Webster Grove, MO 63119-4954 (US).

(74) Agents: BERKSTRESSER, Jerry, W. et al.; Shoemaker and Mattare, Ltd., Suite 1203, Crystal Plaza Building 1, 2001 Jefferson Davis Highway, Arlington, VA 22202-0286 (US).

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(54) Title: HIGH MOLECULAR WEIGHT SURFACE PROTEINS OF NON-TYPEABLE HAEMOPHILUS

(57) Abstract

High molecular weight surface proteins of non-typeable *Haemophilus influenzae* which exhibit immunogenic properties and genes encoding the same are described. Specifically, genes coding for two immunodominant high molecular weight proteins, HMW1 and HMW2, have been cloned, expressed and sequenced, while genes coding for high molecular proteins HMW3 and HMW4 have also been cloned, expressed and sequenced.

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TITLE OF INVENTION

HIGH MOLECULAR WEIGHT SURFACE PROTEINS OF NON-TYPEABLE HAEMOPHILUS

FIELD OF INVENTION

This invention relates to high molecular weight proteins of non-typeable haemophilus.

BACKGROUND TO THE INVENTION

Non-typeable Haemophilus influenzae are non-encapsulated organisms that are defined by their lack of reactivity with antisera against known H. influenzae capsular antigens.

These organisms commonly inhabit the upper respiratory tract of humans and are frequently responsible for a variety of common mucosal surface infections, such as otitis media, sinusitis, conjunctivitis, chronic bronchitis and pneumonia. Otitis media remains an important health problem for children and most children have had at least one episode of otitis by their third birthday and approximately one-third of children have had three or more episodes. Non-typeable Haemophilus influenzae generally accounts for about 20 to 25% of acute otitis media and for a larger percentage of cases of chronic otitis media with effusion.

A critical first step in the pathogenesis of these infections is colonization of the respiratory tract mucosa. Bacterial surface molecules which mediate adherence, therefore, are of particular interest as possible vaccine candidates.

Since the non-typeable organisms do not have a polysaccharide capsule, they are not controlled by the

present Haemophilus influenzae type b (Hib) vaccines, which are directed towards Hib bacterial capsular polysaccharides. The non-typeable strains, however, do produce surface antigens that can elicit bactericidal antibodies. Two of the major outer membrane proteins, P2 and P6, have been identified as targets of human serum bactericidal activity. However, it has been shown that the P2 protein sequence is variable, in particular in the non-typeable Haemophilus strains. Thus, a P2-based vaccine would not protect against all strains of the organism.

There have previously been identified by Barenkamp et al (Pediatr. Infect. Dis. J., 9:333-339, 1990) a group of high-molecular-weight (HMW) proteins of non-typeable Haemophilus influenzae that appeared to be major targets of antibodies present in human convalescent sera. Examination of a series of middle ear isolates revealed the presence of one or two such proteins in most strains. However, prior to the present invention, the structures of these proteins and their encoding nucleic acid sequences were unknown as were pure isolates of such proteins. In addition, the identification of surface accessible epitopes of such proteins was unknown.

SUMMARY OF INVENTION

The inventor, in an effort to further characterize the high molecular weight (HMW) non-typeable Haemophilus proteins, has cloned, expressed and sequenced the genes coding for two immunodominant HMW proteins (designated HMW1 and HMW2) from a prototype non-typeable Haemophilus strain and has cloned, expressed and sequenced the genes coding for two additional immunodominant HMW proteins (designated HMW3 and HMW4) from another non-typeable Haemophilus strain.

In accordance with one aspect of the present invention, therefore, there is provided an isolated and

purified nucleic acid molecule coding for a high molecular weight protein of a non-typeable Haemophilus strain, particularly a nucleic acid molecule coding for protein HMW1, HMW2, HMW3 or HMW4, as well as any variant or fragment of such protein which retains the immunological ability to protect against disease caused by a non-typeable Haemophilus strain.

The nucleic acid molecule may have a DNA sequence shown in Figure 1 (SEQ ID No: 1) and encoding HMW1 for strain 12 having the derived amino acid sequence of Figure 2 (SEQ ID No: 2). The nucleic acid molecule may have the DNA sequence shown in Figure 3 (SEQ ID No: 3) and encoding protein HMW2 for strain 12 having the derived amino acid sequence of Figure 4 (SEQ ID No: 4). The nucleic acid molecule may have the DNA sequence shown in Figure 8 (SEQ ID No: 7) and encoding HMW3 for strain 5 having the derived amino acid sequence of Figure 10 (SEQ ID No: 9). The nucleic acid molecule may have a DNA sequence shown in Figure 9 (SEQ ID No: 8) and encoding protein HMW4 for strain 5 having the derived amino acid sequence of Figure 10 (SEQ ID No: 10).

In another aspect of the invention, there is provided an isolated and purified nucleic acid molecule encoding a high molecular weight protein of a non-typeable Haemophilus strain, which is selected from the group consisting of:

(a) a DNA sequence as shown in any one of Figures 1, 3, 8 and 9 (SEQ ID Nos: 1, 3, 7 and 8);

(b) a DNA sequence encoding an amino acid sequence as shown in any one of Figures 2, 4 and 10 (SEQ ID Nos: 2, 4, 9 and 10); and

(c) a DNA sequence which hybridizes under stringent conditions to any one of the sequences of (a) and (b).

A DNA sequence according to (c) may be one having at least about 90% identity of sequence to the DNA sequences (a) or (b).

The inventor has further found correct processing of the HMW protein requires the presence of additional downstream nucleic acid sequences. Accordingly, a further aspect of the present invention provides an isolated and purified gene cluster comprising a first nucleotide sequence encoding a high molecular weight protein of a non-typeable Haemophilus strain and at least one downstream nucleotide sequence for effecting expression of a gene product of the first nucleotide sequence fully encoded by the structural gene.

The gene cluster may comprise a DNA sequence encoding high molecular weight protein HMW1 or HMW2 and two downstream accessory genes. The gene cluster may have the DNA sequence shown in Figure 6 (SEQ ID No: 5) or Figure 7 (SEQ ID No. 6).

In an additional aspect, the present invention includes a vector adapted for transformation of a host, comprising a nucleic acid molecule as provided herein, particularly the gene cluster provided herein. The vector may be an expression vector or a plasmid adapted for expression of the encoded high molecular weight protein, fragments or analogs thereof, in a heterologous or homologous host and comprising expression means operatively coupled to the nucleic acid molecule. The expression means may include a nucleic acid portion encoding a leader sequence for secretion from the host of the high molecular weight protein. The expression means may include a nucleic acid portion encoding a lipidation signal for expression from the host of a lipidated form of the high molecular weight protein. The host may be selected from, for example, E. coli, Bacillus, Haemophilus, fungi, yeast, baculovirus and Semliki Forest Virus expression systems. The invention further includes

a recombinant high molecular weight protein of non-typeable Haemophilus or fragment or analog thereof producible by the transformed host.

In another aspect, the invention provides an isolated and purified high molecular weight protein of non-typeable Haemophilus influenzae which is encoded by a nucleic acid molecule as provided herein. Such high molecular weight proteins may be produced recombinantly to be devoid of non-high molecular weight proteins of non-typeable Haemophilus influenzae or from natural sources.

Such protein may be characterized by at least one surface-exposed B-cell epitope which is recognized by monoclonal antibody AD6 (ATCC _______). Such protein may be HMW1 encoded by the DNA sequence shown in Figure 1 (SEQ ID No: 1) and having the derived amino acid sequence of Figure 2 (SEQ ID No: 2) and having an apparent molecular weight of 125 kDa. Such protein may be HMW2 encoded by the DNA sequence shown in Figure 3 (SEQ ID No: 3) and having the derived amino acid sequence of Figure 4 (SEQ ID No: 4) and having an apparent molecular weight of 120 kDA. Such protein may be HMW3 encoded by the DNA sequence shown in Figure 8 (SEQ ID No: 7) and having the derived amino acid sequence of Figure 10 (SEQ ID No: 9) and having an apparent molecular weight of 125 kDa. Such protein may be HMW4 encoded by the DNA sequence shown in Figure 9 (SEQ ID No: 8) and having the derived amino acid sequence shown in Figure 10 (SEQ ID No: 10) and having the apparent molecular weight of 123kDa.

A further aspect of the invention provides an isolated and purified high molecular weight protein of non-typeable Haemophilus influenzae which is antigenically related to the filamentous hemagglutinin surface protein of Bordetella pertussis, particularly HMW1, HMW2, HMW3 or HMW4.

The novel high molecular weight proteins of non-typeable Haemophilus may be used as carrier molecules by linking to an antigen, hapten or polysaccharide for eliciting an immune response to the antigen, hapten or polysaccharide. An example of such polysaccharide is a protective polysaccharide against Haemophilus influenzae type b.

In a further aspect of the invention, there is provided a synthetic peptide having an amino acid sequence containing at least six amino acids and no more than 150 amino acids and corresponding to at least one protective epitope of a high molecular weight protein of non-typeable Haemophilus influenzae, specifically HMW1, HMW2, HMW3 or HMW4. The epitope may be one recognized by at least one of the monoclonal antibodies AD6 (ATCC ___) and 10C5 (ATCC _____). Specifically, the epitope may be located within 75 amino acids of the carboxy terminus of the HMW1 or HMW2 protein and recognized by the monoclonal antibody AD6.

The present invention also provides an immunogenic composition comprising an immunoeffective amount of an active component, which may be the novel high molecular weight protein or synthetic peptide provided herein, which may be formulated along with a pharmaceutically acceptable carrier therefor. The immunogenic composition may be formulated as a vaccine for *in vivo* administration to a host.

The immunogenic composition may be formulated as a microparticle, capsule, ISCOM or liposome preparation. The immunogenic composition may be used in combination with a targeting molecule for delivery to specific cells of the immune system or to mucosal surfaces. Some targeting molecules include vitamin B12 and fragments of bacterial toxins, as described in WO 92/17167 (Biotech Australia Pty. Ltd.), and monoclonal antibodies, as described in U.S. Patent No. 5,194,254 (Barber et al).

The immunogenic compositions of the invention (including vaccines) may further comprise at least one other immunogenic or immunostimulating material and the immunostimulating material may be at least one adjuvant.

Suitable adjuvants for use in the present invention include, (but are not limited to) aluminum phosphate, aluminum hydroxide, QS21, Quil A, derivatives and components thereof, ISCOM matrix, calcium phosphate, calcium hydroxide, zinc hydroxide, a glycolipid analog, an octadecyl ester of an amino acid, a muramyl dipeptide polyphosphazare, ISCOPRP, DC-chol, DDBA and a lipoprotein and other adjuvants to induce a Th1 response. Advantageous combinations of adjuvants are described in copending United States patent Application Serial No. 08/261,194 filed June 16, 1994, assigned to Connaught Laboratories Limited and the disclosure of which is incorporated herein by reference.

In a further aspect of the invention, there is provided a method of generating an immune response in a host, comprising administering thereto an immuno-effective amount of the immunogenic composition as provided herein. The immune response may be a humoral or a cell-mediated immune response. Hosts in which protection against disease may be conferred include primates including humans.

The present invention additionally provides a method of producing antibodies specific for a high molecular weight protein of non-typeable Haemophilus influenzae, comprising:

(a) administering the high molecular weight protein or epitope containing peptide provided herein to at least one mouse to produce at least one immunized mouse;

(b) removing B-lymphocytes from the at least one immunized mouse;

(c) fusing the B-lymphocytes from the at least one immunized mouse with myeloma cells, thereby producing hybridomas;

(d) cloning the hybridomas;

(e) selecting clones which produce anti-high molecular weight protein antibody;

(f) culturing the anti-high molecular weight protein antibody-producing clones; and then

(g) isolating anti-high molecular weight protein antibodies from the cultures.

Additional aspects of the present invention include monoclonal antibody AD6 and monoclonal antibody 10C5.

The present invention provides, in an additional aspect thereof, a method for producing an immunogenic composition, comprising administering the immunogenic composition provided herein to a first test host to determine an amount and a frequency of administration thereof to elicit a selected immune response against a high molecular weight protein of non-typeable Haemophilus influenzae; and formulating the immunogenic composition in a form suitable for administration to a second host in accordance with the determined amount and frequency of administration. The second host may be a human.

The novel envelope protein provided herein is useful in diagnostic procedures and kits for detecting antibodies to high molecular weight proteins of non-typeable Haemophilus influenzae. Further monoclonal antibodies specific for the high molecular protein or epitopes thereof are useful in diagnostic procedure and kits for detecting the presence of the high molecular weight protein.

Accordingly, a further aspect of the invention provides a method of determining the presence in a sample, of antibodies specifically reactive with a high molecular weight protein of Haemophilus influenzae comprising the steps of:

(a) contacting the sample with the high molecular weight protein or epitope-containing peptide as provided herein to produce complexes comprising the protein and any said antibodies present in the sample specifically reactive therewith; and

(b) determining production of the complexes.

In a further aspect of the invention, there is provided a method of determining the presence, in a sample, of a high molecular weight protein of Haemophilus influenzae or an epitope-containing peptide, comprising the steps of:

(a) immunizing a host with the protein or peptide as provided herein, to produce antibodies specific for the protein or peptide;

(b) contacting the sample with the antibodies to produce complexes comprising any high molecular weight protein or epitope-containing peptide present in the sample and said specific antibodies; and

(c) determining production of the complexes.

A further aspect of the invention provides a diagnostic kit for determining the presence of antibodies in a sample specifically reactive with a high molecular weight protein of non-typeable Haemophilus influenzae or epitope-containing peptide, comprising:

(a) the high molecular weight protein or epitope-containing peptide as provided herein;

(b) means for contacting the protein or peptide with the sample to produce complexes comprising the protein or peptide and any said antibodies present in the sample; and

(c) means for determining production of the complexes.

The invention also provides a diagnostic kit for detecting the presence, in a sample, of a high molecular weight protein of Haemophilus influenzae or epitope-containing peptide, comprising:

(a) an antibody specific for the novel envelope protein as provided herein;

(b) means for contacting the antibody with the sample to produce a complex comprising the protein or peptide and protein-specific antibody; and

(c) means for determining production of the complex.

In this application, the term "high molecular weight protein" is used to define a family of high molecular weight proteins of Haemophilus influenzae, generally having an apparent molecular weight of from about 120 to about 130 kDa and includes proteins having variations in their amino acid sequences. In this application, a first protein or peptide is a "functional analog" of a second protein or peptide if the first protein or peptide is immunologically related to and/or has the same function as the second protein or peptide. The functional analog may be, for example, a fragment of the protein or a substitution, addition or deletion mutant thereof. The invention also extends to such functional analogs.

Advantages of the present invention include:

- an isolated and purified envelope high molecular weight protein of Haemophilus influenzae produced recombinantly to be devoid of non-high molecular weight proteins of Haemophilus influenzae or from natural sources as well as nucleic acid molecules encoding the same;
- high molecular weight protein specific human monoclonal antibodies which recognize conserved epitopes in such protein; and
- diagnostic kits and immunological reagents for specific identification of hosts infected by Haemophilus influenzae.

BRIEF DESCRIPTION OF DRAWINGS

Figures 1A to 1G contain the DNA sequence of a gene coding for protein HMW1 (SEQ ID No: 1). The hmw1A open reading frame extends from nucleotides 351 to 4958;

Figures 2A and 2B contain the derived amino acid sequence of protein HMW1 (SEQ ID No: 2);

Figures 3A to 3G contain the DNA sequence of a gene coding for protein HMW2 (SEQ ID No: 3). The open hmw2A open reading frame extends from nucleotides 382 to 4782;

Figures 4A and 4B contain the derived amino acid sequence of HMW2 (SEQ ID No: 4);

Figure 5A shows restriction maps of representative recombinant phages which contained the HMW1 or HMW2 structural genes and of HMW1 plasmid subclones. The shaded boxes indicate the location of the structural genes. In the recombinant phage, transcription proceeds from left to right for the HMW1 gene and from right to left for the HMW2 gene;

Figure 5B shows the restriction map of the T7 expression vector pT7-7. This vector contains the T7 RNA polymerase promoter Φ10, a ribosomal binding site (rbs) and the translational start site for the T7 gene 10 protein upstream from a multiple cloning site;

Figures 6A to 6L contain the DNA sequence of a gene cluster for the hmw1 gene (SEQ ID NO: 5), comprising nucleotides 351 to 4958 (ORF a) (as in Figure 1), as well as two additional downstream genes in the 3′ flanking region, comprising ORFs b, nucleotides 5114 to 6748 and c nucleotides 7062 to 9011;

Figures 7A to 7L contain the DNA sequence of a gene cluster for the hmw2 gene (SEQ ID NO: 6), comprising nucleotides 792 to 5222 (ORF a) (as in Figure 3), as well as two additional downstream genes in the 3′ flanking region, comprising ORFs b, nucleotides 5375 to 7009, and c, nucleotides 7249 to 9198;

Figures 8A and 8B contain the DNA sequence of a gene coding for protein HMW3 (SEQ ID NO: 7);

Figures 9A and 9B contain the DNA sequence of a gene coding for protein HMW4 (SEQ ID NO: 8);

Figures 10A to 10L contain a comparison table for the derived amino acid sequence for proteins HMW1 (SEQ ID No: 2), HMW2 (SEQ ID No: 4), HMW3 (SEQ ID No: 9) and HMW4 (SEQ ID No: 10);

Figure 11 illustrates a Western immunoblot assay of phage lysates containing either the HMW1 or HMW2 recombinant proteins. Lysates were probed with an E. coli-absorbed adult serum sample with high-titer antibody against high molecular weight proteins. The arrows indicate the major immunoreactive bands of 125 and 120 kDa in the HMW1 and HMW2 lysates respectively;

Figure 12 is a Western immunoblot assay of cell sonicates prepared from E. coli transformed with plasmid pT7-7 (lanes 1 and 2), pHMW1-2 (lanes 3 and 4), pHMW1-4 (lanes 5 and 6) or pHMW1-14 (lanes 7 and 8). The sonicates were probed with an E. coli-absorbed adult serum sample with high-titer antibody against high-molecular weight proteins. Lanes labelled U and I sequence sonicates prepared before and after indication of the growing samples with IPTG, respectively. The arrows indicate protein bands of interest as discussed below;

Figure 13 is a graphical illustration of an ELISA with rHMW1 antiserum assayed against purified filamentous haemagglutinin of B. pertussis. Ab = antibody;

Figure 14 is a Western immunoblot assay of cell sonicates from a panel of epidemiologically unrelated non-typeable H. influenzae strains. The sonicates were probed with rabbit antiserum prepared against HMW1-4 recombinant protein. The strain designations are indicated by the numbers below each line;

Figure 15 is a Western immunoblot assay of cell sonicates from a panel of epidemiologically unrelated non-typeable H. influenzae strains. The sonicates were probed with monoclonal antibody X3C, a murine 1gG antibody which recognizes the filamentous hemagglutinin of B. pertussis. The strain designations are indicated by the numbers below each line;

Figure 16 shows an immunoblot assay of cell sonicates of non-typeable H. influenzae strain 12 derivatives. The sonicates were probed with rabbit antiserum prepared against HMW-1 recombinant protein. Lanes: 1, wild-type strain; 2, HMW2$^-$ mutant; 3, HMW1$^-$ mutant; 4. HMW1$^-$ HMW2$^-$ double mutant;

Figure 17 shows middle ear bacterial counts in PBS-immunized control animals (left panel) and HMW1/HMW2-immunized animals (right panel) seven days after middle ear inoculation with non-typeable Haemophilus influenzae strain 12. Data are log-transformed and the horizontal lanes indicate the means and standard deviations of middle ear fluid bacterial counts for only the infected animals in each group;

Figure 18 is a schematic diagram of pGEMEX®-hmw1 recombinant plasmids. The restriction enzymes are B-BamHI, E-EcoRI, C-ClaI, RV-EcoRV, Bst-BstEII and H-HindIII;

Figure 19 is a schematic diagram of pGEMEX®-hmw2 recombinant plasmids. The restriction enzymes are E-EcoRI, H-HindIII, Hc-HincII, M-MluI and X-XhoI;

Figure 20 is an immunoelectron micrograph of representative non-typeable Haemophilus influenzae strains after incubation with monoclonal antibody AD6 followed by incubation with goat anti-mouse IgG conjugated with 10-nm colloidal gold particles. Strains are: upper left panel-strain 12; upper right panel-strain 12 mutant deficient in expression of the high molecular

weight proteins; lower left panel-strain 5; lower right panel-strain 15;

Figure 21 is a Western immunoblot assay with Mab AD6 and HMW1 or HMW2 recombinant proteins. The upper left panel indicates the segments of hmw1A or hmw2A structural genes which are being expressed in the recombinant proteins. The lane numbers correspond to the indicated segments;

Figure 22 is a Western immunoblot assay with MAb 10C5 and HMW1 or HMW2 recombinant proteins. The upper panel indicates the segments of the hmw1A or hmw2A structural genes which are being expressed in the recombinant proteins. The lane numbers correspond to the indicated segments; and

Figure 23 is a Western immunoblot assay with MAb AD6 and a panel of unrelated non-typeable Haemophilus influenzae strains which express HMW1/HMW-2 like protein. Cell sonicates were prepared from freshly grown samples of each strain prior to analysis in the Western blot.

GENERAL DESCRIPTION OF INVENTION

The DNA sequences of the genes coding for the HMW1 and HMW2 proteins of non-typeable Haemophilus influenzae strain 12, shown in Figures 1 and 3 respectively, were shown to be about 80% identical, with the first 1259 base pairs of the genes being identical. The open reading frame extend from nucleotides 351 to 4958 and from nucleotide 382 to 4782 respectively. The derived amino acid sequences of the two HMW proteins, shown in Figures 2 and 4 respectively, are about 70% identical. Furthermore, the encoded proteins are antigenically related to the filamentous hemagglutinin surface protein of Bordetella pertussis. A monoclonal antibody prepared against filamentous hemagglutinin (FHA) of Bordetella pertussis was found to recognize both of the high molecular weight proteins. This data suggests that the

HMW and FHA proteins may serve similar biological functions. The derived amino acid sequences of the HMW1 and HMW2 proteins show sequence similarity to that for the FHA protein. It has further been shown that these antigenically-related proteins are produced by the majority of the non-typeable strains of Haemophilus. Antisera raised against the protein expressed by the HMW1 gene recognizes both the HMW2 protein and the B. pertussis FHA. The present invention includes an isolated and purified high molecular weight protein of non-typeable haemophilus which is antigenically related to the B. pertussis FHA and which may be obtained from natural sources or produced recombinantly.

A phage genomic library of a known strain of non-typeable Haemophilus was prepared by standard methods and the library was screened for clones expressing high molecular weight proteins, using a high titre antiserum against HMW's. A number of strongly reactive DNA clones were plaque-purified and sub-cloned into a T7 expression plasmid. It was found that they all expressed either one or the other of the two high-molecular-weight proteins designated HMW1 and HMW2, with apparent molecular weights of 125 and 120 kDa, respectively, encoded by open reading frames of 4.6 kb and 4.4 kb, respectively.

Representative clones expressing either HMW1 or HMW2 were further characterized and the genes isolated, purified and sequenced. The DNA sequence of HMW1 is shown in Figure 1 and the corresponding derived amino acid sequence in Figure 2. Similarly, the DNA sequence of HMW2 is shown in Figure 3 and the corresponding derived amino acid sequence in Figure 4. Partial purification of the isolated proteins and N-terminal sequence analysis indicated that the expressed proteins are truncated since their sequence starts at residue number 442 of both full length HMW1 and HMW2 gene products.

Subcloning studies with respect to the hmw1 and hmw2 genes indicated that correct processing of the HMW proteins required the products of additional downstream genes. It has been found that both the hmw1 and hmw2 genes are flanked by two additional downstream open reading frames (ORFs), designated b and c, respectively, (see Figures 6 and 7).

The b ORFs are 1635 bp in length, extending from nucleotides 5114 to 6748 in the case of hmw1 and nucleotides 5375 to 7009 in the case of hmw2, with their derived amino acid sequences being 99% identical. The derived amino acid sequences demonstrate similarity with the derived amino acid sequences of two genes which encode proteins required for secretion and activation of hemolysins of P. mirabilis and S. marcescens.

The c ORFs are 1950 bp in length, extending from nucleotides 7062 to 9011 in the case of hmw1 and nucleotides 7249 to 9198 in the case of hmw2, with their derived amino acid sequences 96% identical. The hmw1 c ORF is preceded by a series of 9 bp direct tandem repeats. In plasmid subclones, interruption of the hmw1 b or c ORF results in defective processing and secretion of the hmw1 structural gene product.

The two high molecular weight proteins HMW1 and HMW2 have been isolated and purified by the procedures described below in the Examples and shown to be protective against otitis media in chinchillas and to function as adhesins. These results indicate the potential for use of such high molecular proteins and structurally-related proteins of other non-typeable strains of Haemophilus influenzae as components in immunogenic compositions for protecting a susceptible host, such as a human infant, against disease caused by infection with non-typeable Haemophilus influenzae.

Since the proteins provided herein are good cross-reactive antigens and are present in the majority

of non-typeable Haemophilus strains, it is evident that these HMW proteins may become integral constituents of a universal Haemophilus vaccine. Indeed, these proteins may be used not only as protective antigens against otitis, sinusitis and bronchitis caused by the non-typeable Haemophilus strains, but also may be used as carriers for the protective Hib polysaccharides in a conjugate vaccine against meningitis. The proteins also may be used as carriers for other antigens, haptens and polysaccharides from other organisms, so as to induce immunity to such antigens, haptens and polysaccharides.

The nucleotide sequences encoding two high molecular weight proteins of a different non-typeable Haemophilus strain (designated HMW3 and HMW4), namely strain 5 have been elucidated, and are presented in Figures 8 and 9 (SEQ ID Nos: 7 and 8). HMW3 has an apparent molecular weight of 125 kDa while HMW4 has an apparent molecular weight of 123 kDa. These high molecular weight proteins are antigenically related to the HMW1 and HMW2 proteins and to FHA. Figure 10 contains a multiple sequence comparison of the derived amino acid sequences for the four high molecular weight proteins identified herein (HMW1, SEQ ID No: 2; HMW2, SEQ ID No: 4; HMW3, SEQ ID No: 9; HMW4, SEQ ID No. 10). As may be seen from this comparison, stretches of identical amino acid sequence may be found throughout the length of the comparison, with HMW3 more closely resembling HMW1 and HMW4 more closely resembling HMW2. This information is highly suggestive of a considerable sequence homology between high molecular weight proteins from various non-typeable Haemophilus strains. This information is also suggestive that the HMW3 and HMW4 proteins will have the same immunological properties as the HMW1 and HMW2 proteins and that corresponding HMW proteins from other non-typeable Haemophilus strains will have the same immunological properties as the HMW1 and HMW2 proteins.

In addition, mutants of non-typeable H. influenzae strains that are deficient in expression of HMW1 or HMW2 or both have been constructed and examined for their capacity to adhere to cultured human epithelial cells. The hmw1 and hmw2 gene clusters have been expressed in E. coli and have been examined for in vitro adherence. The results of such experimentation, described below, demonstrate that both HMW1 and HMW2 mediate attachment and hence are adhesins and that this function is present even in the absence of other H. influenzae surface structures. The ability of a bacterial surface protein to function as an adhesin provides strong in vitro evidence for its potential role as a protective antigen. In view of the considerable sequence homology between the HMW3 and HMW4 proteins and the HMW1 and HMW2 proteins, these results indicate that HMW3 and HMW4 also are likely to function as adhesins and that other HMW proteins of other strains of non-typeable Haemophilus influenzae similarly are likely to function as adhesins. This expectation is borne out by the results described in the Examples below.

With the isolation and purification of the high molecular weight proteins, the inventor is able to determine the major protective epitopes of the proteins by conventional epitope mapping and synthesizing peptides corresponding to these determinants for incorporation into fully synthetic or recombinant vaccines. Accordingly, the invention also comprises a synthetic peptide having at least six and no more than 150 amino acids and having an amino acid sequence corresponding to at least one protective epitope of a high molecular weight protein of a non-typeable Haemophilus influenzae. Such peptides are of varying length that constitute portions of the high molecular weight proteins, that can be used to induce immunity, either directly or as part of a conjugate, against the respective organisms and thus

constitute active components of immunogenic compositions for protection against the corresponding diseases.

In particular, the applicant has sought to identify regions of the high molecular weight proteins which are demonstrated experimentally to be surface-exposed B-cell epitopes and which are common to all or at least a large number of non-typeable strains of Haemophilus influenzae. The strategy which has been adopted by the inventor has been to:

(a) generate a panel of monoclonal antibodies reactive with the high molecular weight proteins;

(b) screen those monoclonal antibodies for reactivity with surface epitopes of intact bacteria using immunoelectron microscopy or other suitable screening technique;

(c) map the epitopes recognized by the monoclonal antibody by determining the reactivity of the monoclonals with a panel of recombinant fusion proteins; and

(d) determining the reactivity of the monoclonal antibodies with heterologous non-typable Haemophilus influenzae strains using standard Western blot assays.

Using this approach, the inventor has identified one monoclonal antibody, designated AD6 (ATCC ______), which recognized a surface-exposed B-cell epitope common to all non-typeable H. influenzae which express the HMW1 and HMW2 proteins. The epitope recognized by this antibody was mapped to a 75 amino acid sequence at the carboxy termini of both HMW1 and HMW2 proteins. The ability to identify shared surface-exposed epitopes on the high molecular weight adhesion proteins suggests that it would be possible to develop recombinant or synthetic peptide based vaccines which would be protective against disease caused by the majority of non-typeable Haemophilus influenzae.

The present invention also provides any variant or fragment of the proteins that retains the potential immunological ability to protect against disease caused by non-typeable Haemophilus strains. The variants may be constructed by partial deletions or mutations of the genes and expression of the resulting modified genes to give the protein variants.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis, treatment of bacterial infections and the generation of immunological reagents. A further non-limiting discussion of such uses is further presented below.

1. Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as vaccines, may be prepared from the high molecular weight proteins of Haemophilus influenzae, as well as analogs and fragments thereof, and synthetic peptides containing epitopes of the protein, as disclosed herein. The immunogenic composition elicits an immune response which produces antibodies, including anti-high molecular weight protein antibodies and antibodies that are opsonizing or bactericidal.

Immunogenic compositions, including vaccines, may be prepared as injectables, as liquid solutions or emulsions. The active component may be mixed with pharmaceutically acceptable excipients which are compatible therewith. Such excipients may include, water, saline, dextrose, glycerol, ethanol, and combinations thereof. The immunogenic compositions and vaccines may further contain auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness thereof. Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously or

intramuscularly. Alternatively, the immunogenic compositions formed according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces. Thus, the immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral (intragastric) routes. Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example, polyalkalene glycols or triglycerides. Oral formulations may include normally employed incipients such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate. These compositions can take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain about 1 to 95% of the active component. The immunogenic preparations and vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize antibodies, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of micrograms of the HMW proteins. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage may also depend on the route of administration and will vary according to the size of the host.

The concentration of the active component in an immunogenic composition according to the invention is in

general about 1 to 95%. A vaccine which contains antigenic material of only one pathogen is a monovalent vaccine. Vaccines which contain antigenic material of several pathogens are combined vaccines and also belong to the present invention. Such combined vaccines contain, for example, material from various pathogens or from various strains of the same pathogen, or from combinations of various pathogens.

Immunogenicity can be significantly improved if the antigens are co-administered with adjuvants, commonly used as 0.05 to 0.1 percent solution in phosphate-buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Intrinsic adjuvants, such as lipopolysaccharides, normally are the components of the killed or attenuated bacteria used as vaccines. Extrinsic adjuvants are immunomodulators which are typically non-covalently linked to antigens and are formulated to enhance the host immune responses. Thus, adjuvants have been identified that enhance the immune response to antigens delivered parenterally. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. The efficacy of alum in

increasing antibody responses to diphtheria and tetanus toxoids is well established and a HBsAg vaccine has been adjuvanted with alum. While the usefulness of alum is well established for some applications, it has limitations. For example, alum is ineffective for influenza vaccination and inconsistently elicits a cell mediated immune response. The antibodies elicited by alum-adjuvanted antigens are mainly of the IgG1 isotype in the mouse, which may not be optimal for protection by some vaccinal agents.

A wide range of extrinsic adjuvants can provoke potent immune responses to antigens. These include saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes.

To efficiently induce humoral immune responses (HIR) and cell-mediated immunity (CMI), immunogens are often emulsified in adjuvants. Many adjuvants are toxic, inducing granulomas, acute and chronic inflammations (Freund's complete adjuvant, FCA), cytolysis (saponins and Pluronic polymers) and pyrogenicity, arthritis and anterior uveitis (LPS and MDP). Although FCA is an excellent adjuvant and widely used in research, it is not licensed for use in human or veterinary vaccines because of its toxicity.

Desirable characteristics of ideal adjuvants include:

(1) lack of toxicity;

(2) ability to stimulate a long-lasting immune response;

(3) simplicity of manufacture and stability in long-term storage;

(4) ability to elicit both CMI and HIR to antigens administered by various routes, if required;

(5) synergy with other adjuvants;

(6) capability of selectively interacting with populations of antigen presenting cells (APC);

(7) ability to specifically elicit appropriate T_H1 or T_H2 cell-specific immune responses; and

(8) ability to selectively increase appropriate antibody isotype levels (for example, IgA) against antigens.

U.S. Patent No. 4,855,283 granted to Lockhoff et al on August 8, 1989 which is incorporated herein by reference thereto teaches glycolipid analogues including N-glycosylamides, N-glycosylureas and N-glycosylcarbamates, each of which is substituted in the sugar residue by an amino acid, as immuno-modulators or adjuvants. Thus, Lockhoff et al. (US Patent No. 4,855,283 and ref. 29) reported that N-glycolipid analogs displaying structural similarities to the naturally-occurring glycolipids, such as glycosphingolipids and glycoglycerolipids, are capable of eliciting strong immune responses in both herpes simplex virus vaccine and pseudorabies virus vaccine. Some glycolipids have been synthesized from long chain-alkylamines and fatty acids that are linked directly with the sugars through the anomeric carbon atom, to mimic the functions of the naturally occurring lipid residues.

U.S. Patent No. 4,258,029 granted to Moloney, incorporated herein by reference thereto, teaches that octadecyl tyrosine hydrochloride (OTH) functioned as an adjuvant when complexed with tetanus toxoid and formalin inactivated type I, II and III poliomyelitis virus vaccine. Also, Nixon-George et al. (ref. 30), reported that octadecyl esters of aromatic amino acids complexed with a recombinant hepatitis B surface antigen, enhanced the host immune responses against hepatitis B virus.

Lipidation of synthetic peptides has also been used to increase their immunogenicity. Thus, Wiesmuller 1989, describes a peptide with a sequence homologous to a foot-

and-mouth disease viral protein coupled to an adjuvant tripalmityl-s-glyceryl-cysteinylserylserine, being a synthetic analogue of the N-terminal part of the lipoprotein from Gram negative bacteria. Furthermore, Deres et al. 1989, reported *in vivo* priming of virus-specific cytotoxic T lymphocytes with synthetic lipopeptide vaccine which comprised of modified synthetic peptides derived from influenza virus nucleoprotein by linkage to a lipopeptide, N-palmityl-s-[2,3-bis(palmitylxy)-(2RS)-propyl-[R]-cysteine (TPC).

2. Immunoassays

The high molecular weight protein of Haemophilus influenzae of the present invention is useful as an immunogen for the generation of anti-protein antibodies, as an antigen in immunoassays including enzyme-linked immunosorbent assays (ELISA), RIAs and other non-enzyme linked antibody binding assays or procedures known in the art for the detection of antibodies. In ELISA assays, the protein is immobilized onto a selected surface, for example, a surface capable of binding proteins, such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed protein, a nonspecific protein, such as a solution of bovine serum albumin (BSA) that is known to be antigenically neutral with regard to the test sample, may be bound to the selected surface. This allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of antisera onto the surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This may include diluting the sample with diluents, such as solutions of BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to

incubate for from about 2 to 4 hours, at temperatures such as of the order of about 25° to 37°C. Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution, such as PBS/Tween or a borate buffer. Following formation of specific immunocomplexes between the test sample and the bound protein, and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined by subjecting the immunocomplex to a second antibody having specificity for the first antibody. If the test sample is of human origin, the second antibody is an antibody having specificity for human immunoglobulins and in general IgG. To provide detecting means, the second antibody may have an associated activity such as an enzymatic activity that will generate, for example, a colour development upon incubating with an appropriate chromogenic substrate. Quantification may then be achieved by measuring the degree of colour generation using, for example, a visible spectra spectrophotometer.

3. Use of Sequences as Hybridization Probes

The nucleotide sequences of the present invention, comprising the sequences of the genes encoding the high molecular weight proteins of specific strains of non-typeable Haemophilus influenzae, now allow for the identification and cloning of the genes from any species of non-typeable Haemophilus and other strains of non-typeable Haemophilus influenzae.

The nucleotide sequences comprising the sequences of the genes of the present invention are useful for their ability to selectively form duplex molecules with complementary stretches of other genes of high molecular weight proteins of non-typeable Haemophilus. Depending on the application, a variety of hybridization conditions may be employed to achieve varying degrees of selectivity

of the probe toward the other genes. For a high degree of selectivity, relatively stringent conditions are used to form the duplexes, such as low salt and/or high temperature conditions, such as provided by 0.02 M to 0.15 M NaCl at temperatures of between about 50°C to 70°C. For some applications, less stringent hybridization conditions are required such as 0.15 M to 0.9 M salt, at temperatures ranging from between about 20°C to 55°C. Hybridization conditions can also be rendered more stringent by the addition of increasing amounts of formamide, to destabilize the hybrid duplex. Thus, particular hybridization conditions can be readily manipulated, and will generally be a method of choice depending on the desired results. In general, convenient hybridization temperatures in the presence of 50% formamide are: 42°C for a probe which is 95 to 100% homologous to the target fragment, 37°C for 90 to 95% homology and 32°C for 85 to 90% homology.

In a clinical diagnostic embodiment, the nucleic acid sequences of the genes of the present invention may be used in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of providing a detectable signal. In some diagnostic embodiments, an enzyme tag such as urease, alkaline phosphatase or peroxidase, instead of a radioactive tag may be used. In the case of enzyme tags, colorimetric indicator substrates are known which can be employed to provide a means visible to the human eye or spectrophotometrically, to identify specific hybridization with samples containing gene sequences encoding high molecular weight proteins of non-typeable <u>Haemophilus</u>.

The nucleic acid sequences of genes of the present invention are useful as hybridization probes in solution

hybridizations and in embodiments employing solid-phase procedures. In embodiments involving solid-phase procedures, the test DNA (or RNA) from samples, such as clinical samples, including exudates, body fluids (e. g., serum, amniotic fluid, middle ear effusion, sputum, bronchoalveolar lavage fluid) or even tissues, is adsorbed or otherwise affixed to a selected matrix or surface. The fixed, single-stranded nucleic acid is then subjected to specific hybridization with selected probes comprising the nucleic acid sequences of the genes or fragments thereof of the present invention under desired conditions. The selected conditions will depend on the particular circumstances based on the particular criteria required depending on, for example, the G+C contents, type of target nucleic acid, source of nucleic acid, size of hybridization probe etc. Following washing of the hybridization surface so as to remove non-specifically bound probe molecules, specific hybridization is detected, or even quantified, by means of the label. As with the selection of peptides, it is preferred to select nucleic acid sequence portions which are conserved among species of non-typeable Haemophilus. The selected probe may be at least about 18 bp and may be in the range of about 30 bp to about 90 bp long.

4. Expression of the High Molecular Weight Protein Genes

Plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell may be used for the expression of the genes encoding high molecular weight proteins of non-typeable Haemophilus in expression systems. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* may be transformed using pBR322 which contains genes for ampicillin and tetracycline resistance and thus provides

easy means for identifying transformed cells. The pBR322 plasmid, or other microbial plasmid or phage must also contain, or be modified to contain, promoters which can be used by the host cell for expression of its own proteins.

In addition, phage vectors containing replicon and control sequences that are compatible with the host can be used as a transforming vector in connection with these hosts. For example, the phage in lambda GEM™-11 may be utilized in making recombinant phage vectors which can be used to transform host cells, such as E. coli LE392.

Promoters commonly used in recombinant DNA construction include the β-lactamase (penicillinase) and lactose promoter systems (Chang *et al.*, 1978: Itakura *et al.*, 1977 Goeddel *et al.*, 1979; Goeddel *et al.*, 1980) and other microbial promoters such as the T7 promoter system (U.S. Patent 4,952,496). Details concerning the nucleotide sequences of promoters are known, enabling a skilled worker to ligate them functionally with genes. The particular promoter used will generally be a matter of choice depending upon the desired results. Hosts that are appropriate for expression of the genes encoding the high molecular weight proteins, fragment analogs or variants thereof, include E. coli, Bacillus species, Haemophilus, fungi, yeast or the baculovirus expression system may be used.

In accordance with this invention, it is preferred to make the high molecular weight proteins by recombinant methods, particularly since the naturally occurring high molecular weight protein as purified from a culture of a species of non-typeable Haemophilus may include trace amounts of toxic materials or other contaminants. This problem can be avoided by using recombinantly produced proteins in heterologous systems which can be isolated from the host in a manner to minimize comtaminants in the purified material. Particularly desirable hosts for

expression in this regard include Gram positive bacteria which do not have LPS and are, therefore, endotoxin free. Such hosts include species of Bacillus and may be particularly useful for the production of non-pyrogenic high molecular weight protein, fragments or analogs thereof. Furthermore, recombinant methods of production permit the manufacture of HMW1, HMW2, HMW3 or HMW4, and corresponding HMW proteins from other non-typeable Haemophilus influenzae strains, or fragments thereof, separate from one another and devoid of non-HMW protein of non-typeable Haemophilus influenzae.

Biological Deposits

Certain hybridomas producing monoclonal antibodies specific for high molecular weight protein of Haemophilus influenzae according to aspects of the present invention that are described and referred to herein have been deposited with the American Type Culture Collection (ATCC) located at 12301 Parklawn Drive, Rockville, Maryland, USA, 20852, pursuant to the Budapest Treaty and prior to the filing of this application. Samples of the deposited hybridomas will become available to the public upon grant of a patent based upon this United States patent application. The invention described and claimed herein is not to be limited in scope by the hybridomas deposited, since the deposited embodiment is intended only as an illustration of the invention. Any equivalent or similar hybridomas that produce similar or equivalent antibodies as described in this application are within the scope of the invention.

Deposit Summary

Hybridomas	ATCC Designation	Date Deposited
AD6		
10C5		

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, protein biochemistry, and immunology used but not explicitly described in this disclosure and these Examples are amply reported in the scientific literature and are well within the ability of those skilled in the art.

Example 1:

This Example describes the isolation of DNA encoding HMW1 and HMW2 proteins, cloning and expression of such proteins, and sequencing and sequence analysis of the DNA molecules encoding the HMW1 and HMW2 proteins.

Non-typeable H.influenzae strains 5 and 12 were isolated in pure culture from the middle ear fluid of children with acute otitis media. Chromosomal DNA from strain 12, providing genes encoding proteins HMW1 and HMW2, was prepared by preparing Sau3A partial restriction digests of chromosomal DNA and fractionating on sucrose gradients. Fractions containing DNA fragments in the 9 to 20 kbp range were pooled and a library was prepared by ligation into λEMBL3 arms. Ligation mixtures were packaged in vitro and plate-amplified in a P2 lysogen of E. coli LE392.

For plasmid subcloning studies, DNA from a representative recombinant phage was subcloned into the T7 expression plasmid pT7-7, containing the T7 RNA polymerase promoter Φ10, a ribosome-binding site and the

translational start site for the T7 gene 10 protein upstream from a multiple cloning site (see Figure 5B).

DNA sequence analysis was performed by the dideoxy method and both strands of the HMW1 gene and a single strand of the HMW2 gene were sequenced.

Western immunoblot analysis was performed to identify the recombinant proteins being produced by reactive phage clones (Figure 11). Phage lysates grown in LE392 cells or plaques picked directly from a lawn of LE392 cells on YT plates were solubilized in gel electrophoresis sample buffer prior to electrophoresis. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 7.5% or 11% polyacrylamide modified Laemmli gels. After transfer of the proteins to nitrocellulose sheets, the sheets were probed sequentially with an <u>E. coli</u>-absorbed human serum sample containing high-titer antibody to the high-molecular-weight proteins and then with alkaline phosphatase-conjugated goat anti-human immunoglobulin G (IgG) second antibody. Sera from healthy adults contains high-titer antibody directed against surface-exposed high-molecular-weight proteins of non-typeable <u>H. influenzae</u>. One such serum sample was used as the screening antiserum after having been extensively absorbed with LE392 cells.

To identify recombinant proteins being produced by <u>E. coli</u> transformed with recombinant plasmids, the plasmids of interest were used to transform <u>E. coli</u> BL21 (DE3)/pLysS. The transformed strains were grown to an A_{600} of 0.5 in L broth containing 50 μg of ampicillin per ml. IPTG was then added to 1 mM. One hour later, cells were harvested, and a sonicate of the cells was prepared. The protein concentrations of the samples were determined by the bicinchoninic acid method. Cell sonicates containing 100 μg of total protein were solubilized in electrophoresis sample buffer, subjected to SDS-polyacrylamide gel electrophoresis, and transferred to

nitrocellulose. The nitrocellulose was then probed sequentially with the E. coli-absorbed adult serum sample and then with alkaline phosphatase-conjugated goat anti-human IgG second antibody.

Western immunoblot analysis also was performed to determine whether homologous and heterologous non-typeable H. influenzae strains expressed high-molecular-weight proteins antigenically related to the protein encoded by the cloned HMW1 gene (rHMW1). Cell sonicates of bacterial cells were solubilized in electrophoresis sample buffer, subjected to SDS-polyacrylamide gel electrophoresis, and transferred to nitrocellulose. Nitrocellulose was probed sequentially with polyclonal rabbit rHMW1 antiserum and then with alkaline phosphatase-conjugated goat anti-rabbit IgG second antibody.

Finally, Western immunoblot analysis was performed to determine whether non-typeable Haemophilus strains expressed proteins antigenically related to the filamentous hemagglutinin protein of Bordetella pertussis. Monoclonal antibody X3C, a murine immunoglobulin G (IgG) antibody which recognizes filamentous hemagglutinin, was used to probe cell sonicates by Western blot. An alkaline phosphatase-conjugated goat anti-mouse IgG second antibody was used for detection.

To generate recombinant protein antiserum, E. coli BL21(DE3)/pLysS was transformed with pHMW1-4, and expression of recombinant protein was induced with IPTG, as described above. A cell sonicate of the bacterial cells was prepared and separated into a supernatant and pellet fraction by centrifugation at 10,000 x g for 30 min. The recombinant protein fractionated with the pellet fraction. A rabbit was subcutaneously immunized on biweekly schedule with 1 mg of protein from the pellet fraction, the first dose given with Freund's complete

adjuvant and subsequent doses with Freund's incomplete adjuvant. Following the fourth injection, the rabbit was bled. Prior to use in the Western blot assay, the antiserum was absorbed extensively with sonicates of the host E. coli strain transformed with cloning vector alone.

To assess the sharing of antigenic determinants between HMW1 and filamentous hemagglutinin, enzyme-linked immunosorbent assay (ELISA) plates (Costar, Cambridge, Mass.) were coated with 60 μl of a 4-μg/ml solution of filamentous hemagglutinin in Dulbecco's phosphate-buffered saline per well for 2 h at room temperature. Wells were blocked for 1 h with 1% bovine serum albumin in Dulbecco's phosphate-buffered saline prior to addition of serum dilutions. rHMW1 antiserum was serially diluted in 0.1% Brij (Sigma, St. Louis, Mo.) in Dulbecco's phosphate-buffered saline and incubated for 3 h at room temperature. After being washed, the plates were incubated with peroxidase-conjugated goat anti-rabbit lgG antibody (Bio-Rad) for 2 h at room temperature and subsequently developed with 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma) at a concentration of 0.54 in mg/ml in 0.1 M sodium citrate buffer, pH 4.2, containing 0.03% H_2O_2. Absorbances were read on an automated ELISA reader.

Recombinant phage expressing HMW1 or HMW2 were recovered as follows. The non-typeable H. influenzae strain 12 genomic library was screened for clones expressing high-molecular-weight proteins with an E. coli-absorbed human serum sample containing a high titer of antibodies directed against the high-molecular-weight proteins.

Numerous strongly reactive clones were identified along with more weakly reactive ones. Twenty strongly reactive clones were plaque-purified and examined by Western blot for expression of recombinant proteins.

Each of the strongly reactive clones expressed one of two types of high-molecular-weight proteins, designated HMW1 and HMW2. The major immunoreactive protein bands in the HMW1 and HMW2 lysates migrated with apparent molecular masses of 125 and 120 kDa, respectively. In addition to the major bands, each lysate contained minor protein bands of higher apparent molecular weight. Protein bands seen in the HMW2 lysates at molecular masses of less than 120 kDa were not regularly observed and presumably represent proteolytic degradation products. Lysates of LE392 infected with the λEMBL3 cloning vector alone were non-reactive when immunologically screened with the same serum sample. Thus, the observed activity was not due to cross-reactive E. coli proteins or λEMBL3-encoded proteins. Furthermore, the recombinant proteins were not simply binding immunoglobulin nonspecifically, since the proteins were not reactive with the goat anti-human IgG conjugate alone, with normal rabbit sera, or with serum from a number of healthy young infants.

Representative clones expressing either the HMW1 or HMW2 recombinant proteins were characterized further. The restriction maps of the two phage types were different from each other, including the regions encoding the HMW1 and HMW2 structural genes. Figure 5A shows restriction maps of representative recombinant phage which contained the HMW1 or HMW2 structural genes. The locations of the structural genes are indicated by the shaded bars.

HMW1 plasmid subclones were constructed by using the T7 expression plasmid T7-7 (Fig. 5A and B). HMW2 plasmid subclones also were constructed, and the results with these latter subclones were similar to those observed with the HMW1 constructs.

The approximate location and direction of transcription of the HMW1 structure gene were initially determined by using plasmid pHMW1 (Fig. 5A). This

plasmid was constructed by inserting the 8.5-kb BamHI-SalI fragment from λHMW1 into BamHI- and SalI-cut pT7-7. E. coli transformed with pHMW1 expressed an immunoreactive recombinant protein with an apparent molecular mass of 115 kDa, which was strongly inducible with IPTG. This protein was significantly smaller than the 125-kDa major protein expressed by the parent phage, indicating that it either was being expressed as a fusion protein or was truncated at the carboxy terminus.

To more precisely localize the 3′ end of the structural gene, additional plasmids were constructed with progressive deletions from the 3′ end of the pHMW1 construct. Plasmid pHMW1-1 was constructed by digestion of pHMW1 with PstI, isolation of the resulting 8.8-kb fragment, and religation. Plasmid pHMW1-2 was constructed by digestion of pHMW1 with HindIII, isolation of the resulting 7.5-kb fragment, and religation. E. coli transformed with either plasmid pHMW1-1 or pHMW1-2 also expressed an immunoreactive recombinant protein with an apparent molecular mass of 115 kDa. These results indicated that the 3′ end of the structural gene was 5′ of the HindIII site. Figure 12 demonstrates the Western blot results with pHMW1-2 transformed cells before and after IPTG indicates (lanes 3 and 4, respectively). The 115 kDa recombinant protein is indicated by the arrow. Transformants also demonstrated cross-reactive bands of lower apparent molecular weight, and probably represent partial degradation products. Shown for comparison and the results for E. coli transformed with the pT7-7 cloning vector alone (Fig. 12, lanes 1 and 2).

To more precisely localize the 5′ end of the gene, plasmids pHMW1-4 and pHMW1-7 were constructed. Plasmid pHMW1-4 was constructed by cloning the 5.1-kb BamHI-HindIII fragment from λHMW1 into a pT7-7-derived plasmid containing the upstream 3.8-kb EcoRI-BamHi fragment. E. coli transformed with pHMW1-4 expressed an immunoreactive

protein with an apparent molecular mass of approximately 160 kDa (Fig. 12, lane 6). Although protein production was inducible with IPTG, the levels of protein production in these transformants were substantially lower than those with the pHMW1-2 transformants described above. Plasmid pHMW1-7 was constructed by digesting pHMW1-4 with NdeI and SpeI. The 9.0-kbp fragment generated by this double digestion was isolated, blunt ended, and religated. E. coli transformed with pHMW1-7 also expressed an immunoreactive protein with an apparent molecular mass of 160 kDa, a protein identical in size to that expressed by the pHMW1-4 transformants. The result indicated that the initiation codon for the HMW1 structural gene was 3' of the SpeI site. DNA sequence analysis (described below) confirmed this conclusion.

As noted above, the λHMW1 phage clones expressed a major immunoreactive band of 125 kDa, whereas the HMW1 plasmid clones pHMW1-4 and pHMW1-7, which contained what was believed to be the full-length gene, expressed an immunoreactive protein of approximately 160 kDa. This size discrepancy was disconcerting. One possible explanation was that an additional gene or genes necessary for correct processing of the HMW1 gene product were deleted in the process of subcloning. To address this possibility, plasmid pHMW1-14 was constructed. This construct was generated by digesting pHMW1 with NdeI and MluI and inserting the 7.6-kbp NdeI-MluI fragment isolated from pHMW1-4. Such a construct would contain the full-length HMW1 gene as well as the DNA 3' of the HMW1 gene which was present in the original HMW1 phage. E. coli transformed with this plasmid expressed major immunoreactive proteins with apparent molecular masses of 125 and 160 kDa as well as additional degradation products (Fig. 12, lanes 7 and 8). The 125- and 160-kDa bands were identical to the major and minor immunoreactive bands detected in the HMW1 phage lysates.

Interestingly, the pHMW1-14 construct also expressed significant amounts of protein in the uninduced condition, a situation not observed with the earlier constructs.

The relationship between the 125- and 160-kDa proteins remains somewhat unclear. Sequence analysis, described below, reveals that the HMW1 gene would be predicted to encode a protein of 159 kDa. It is believed that the 160-kDa protein is a precursor form of the mature 125-kDa protein, with the conversion from one protein to the other being dependent on the products of the two downstream genes.

Sequence analysis of the HMW1 gene (Figure 1) revealed a 4,608-bp open reading frame (ORF), beginning with an ATG codon at nucleotide 351 and ending with a TAG stop codon at nucleotide 4959. A putative ribosome-binding site with the sequence AGGAG begins 10 bp up-stream of the putative initiation codon. Five other in-frame ATG codons are located within 250 bp of the beginning of the ORF, but none of these is preceded by a typical ribosome-binding site. The 5′-flanking region of the ORF contains a series of direct tandem repeats, with the 7-bp sequence ATCTTTC repeated 16 times. These tandem repeats stop 100 bp 5′ of the putative initiation codon. An 8-bp inverted repeat characteristic of a rho-independent transcriptional terminator is present, beginning at nucleotide 4983, 25 bp 3′ of the presumed translational stop. Multiple termination codons are present in all three reading frames both upstream and downstream of the ORF. The derived amino acid sequence of the protein encoded by the HMW1 gene (Figure 2) has a molecular weight of 159,000, in good agreement with the apparent molecular weights of the proteins expressed by the HMW1-4 and HMW1-7 transformants. The derived amino acid sequence of the amino terminus does not demonstrate the characteristics of a typical signal sequence. The

BamHI site used in generation of pHMW1 comprises bp 1743 through 1748 of the nucleotide sequence. The ORF downstream of the BamHI site would be predicted to encode a protein of 111 kDa, in good agreement with the 115 kDa estimated for the apparent molecular mass of the pHMW1-encoded fusion protein.

The sequence of the HMW2 gene (Figure 3) consists of a 4,431-bp ORF, beginning with an ATG codon at nucleotide 352 and ending with a TAG stop codon at nucleotide 4783. The first 1,259 bp of the ORF of the HMW2 gene are identical to those of the HMW1 gene. Thereafter, the sequences begin to diverge but are 80% identical overall. With the exception of a single base addition at nucleotide 93 of the HMW2 sequence, the 5′-flanking regions of the HMW1 and HMW2 genes are identical for 310 bp upstream from the respective initiation codons. Thus, the HMW2 gene is preceded by the same set of tandem repeats and the same putative ribosome-binding site which lies 5′ of the HMW1 gene. A putative transcriptional terminator identical to that identified 3′ of the HMW1 ORF is noted, beginning at nucleotide 4804. The discrepancy in the lengths of the two genes is principally accounted for by a 186-bp gap in the HMW2 sequence, beginning at nucleotide position 3839. The derived amino acid sequence of the protein encoded by the HMW2 gene (Figure 4) has a molecular weight of 155,000 and is 71% identical with the derived amino acid sequence of the HMW1 gene.

The derived amino acid sequences of both the HMW1 and HMW2 genes (Figures 2 and 4) demonstrated sequence similarity with the derived amino acid sequence of filamentous hemagglutinin of Bordetella pertussis, a surface-associated protein of this organism. The initial and optimized TFASTA scores for the HMW1-filamentous hemagglutinin sequence comparison were 87 and 186, respectively, with a word size of 2. The z score for the

comparison was 45.8. The initial and optimized TFASTA scores for the HMW2-filamentous hemagglutinin sequence comparison were 68 and 196, respectively. The z score for the latter comparison was 48.7. The magnitudes of the initial and optimized TFASTA scores and the z scores suggested that a biologically significant relationship existed between the HMW1 and HMW2 gene products and filamentous hemagglutinin. When the derived amino acid sequences of HMW1, HMW2, and filamentous hemagglutinin genes were aligned and compared, the similarities were most notable at the amino-terminal ends of the three sequences. Twelve of the first 22 amino acids in the predicted peptide sequences were identical. In addition, the sequences demonstrated a common five-amino-acid stretch, Asn-Pro-Asn-Gly-Ile, and several shorter stretches of sequence identity within the first 200 amino acids.

Example 2:

This Example describes the relationship of filamentous hemagglutinin and the HMW1 protein.

To further explore the HMW1-filamentous hemagglutinin relationship, the ability of antiserum prepared against the HMW1-4 recombinant protein (rHMW1) to recognize purified filamentous hemagglutinin was assessed (Figure 13). The rHMW1 antiserum demonstrated ELISA reactivity with filamentous hemagglutinin in a dose-dependent manner. Preimmune rabbit serum had minimal reactivity in this assay. The rHMW1 antiserum also was examined in a Western blot assay and demonstrated weak but positive reactivity with purified filamentous hemagglutinin in this system also.

To identify the native Haemophilus protein corresponding to the HMW1 gene product and to determine the extent to which proteins antigenically related to the HMW1 cloned gene product were common among other non-typeable H. influenzae strains, a panel of Haemophilus

strains was screened by Western blot with the rHMW1 antiserum. The antiserum recognized both a 125- and a 120-kDa protein band in the homologous strain 12 (Figure 14), the putative mature protein products of the HMW1 and HMW2 genes, respectively. The 120-kDa protein appears as a single band in Figure 14, wherein it appeared as a doublet in the HMW2 phage lysates (Figure 11).

When used to screen heterologous non-typeable H. influenzae strains, rHMW1 antiserum recognized high-molecular-weight proteins in 75% of 125 epidemiologically unrelated strains. In general, the antiserum reacted with one or two protein bands in the 100- to 150-kDa range in each of the heterologous strains in a pattern similar but not identical to that seen in the homologous strain (Figure 14).

Monoclonal antibody X3C is a murine IgG antibody directed against the filamentous hemagglutinin protein of B. pertussis. This antibody can inhibit the binding of B. pertussis cells to Chinese hamster ovary cells and HeLa cells in culture and will inhibit hemagglutination of erythrocytes by purified filamentous hemagglutinin. A Western blot assay was performed in which this monoclonal antibody was screened against the same panel of non-typeable H. influenzae strains discussed above (Figure 14). Monoclonal antibody X3C recognized both the high-molecular-weight proteins in non-typeable H. influenzae strain 12 which were recognized by the recombinant-protein antiserum (Figure 15). In addition, the monoclonal antibody recognized protein bands in a subset of heterologous non-typeable H. influenzae strains which were identical to those recognized by the recombinant-protein antiserum, as may be seen by comparison of Figures 14 and 15. On occasion, the filamentous hemagglutinin monoclonal antibody appeared to recognize only one of the two bands which had been recognized by the recombinant-protein antiserum (compare

strain lane 18 in Figures 14 and 15, for example). Overall, monoclonal antibody X3C recognized high-molecular-weight protein bands identical to those recognized by the rHMW1 antiserum in approximately 35% of our collection of non-typeable H. influenzae strains.

Example 3:

This Example describes the adhesin properties of the HMW1 and HMW2 proteins.

Mutants deficient in expression of HMW1, HMW2 or both proteins were constructed to examine the role of these proteins in bacterial adherence. The following strategy was employed. pHMW1-14 (see Example 1, Figure 5A) was digested with BamHI and then ligated to a kanamycin cassette isolated on a 1.3-kb BamH1 fragment from pUC4K. The resultant plasmid (pHMW1-17) was linearized by digestion with XbaI and transformed into non-typeable H. influenzae strain 12, followed by selection for kanamycin resistant colonies. Southern analysis of a series of these colonies demonstrated two populations of transformants, one with an insertion in the HMW1 structural gene and the other with an insertion in the HMW2 structural gene. One mutant from each of these classes was selected for further studies.

Mutants deficient in expression of both proteins were recovered using the following protocol. After deletion of the 2.1-kb fragment of DNA between two EcoRI sites spanning the 3′-portion of the HMW1 structural gene and the 5′-portion of a downstream gene encoding an accessory processing protein in pHMW-15, the kanamycin cassette from pUC4K was inserted as a 1.3-kb EcoR1 fragment. The resulting plasmid (pHMW1-16) was linearized by digestion with XbaI and transformed into strain 12, followed again by selection for kanamycin resistant colonies. Southern analysis of a representative sampling of these colonies demonstrated that in seven of eight cases, insertion into both the

HMW1 and HMW2 loci had occurred. One such mutant was selected for further studies.

To confirm the intended phenotypes, the mutant strains were examined by Western blot analysis with a polyclonal antiserum against recombinant HMW1 protein. The parental strain expressed both the 125-kD HMW1 and the 120-kD HMW2 protein (Figure 16). In contrast, the HMW2⁻ mutant failed to express the 120-kD protein, and the HMW1 mutant failed to express the 125-kD protein. The double mutant lacked expression of either protein. On the basis of whole cell lysates, outer membrane profiles, and colony morphology, the wild type strain and the mutants were otherwise identical with one another. Transmission electron microscopy demonstrated that none of the four strains expressed pili.

The capacity of wild type strain 12 to adhere to Chang epithelial cells was examined. In such assays, bacteria were inoculated into broth and allowed to grow to a density of ~2 x 10^9 cfu/ml. Approximately 2 x 10^7 cfu were inoculated onto epithelial cell monolayers, and plates were gently centrifuged at 165 x g for 5 minutes to facilitate contact between bacteria and the epithelial surface. After incubation for 30 minutes at 37°C in 5% CO_2, monolayers were rinsed 5 times with PBS to remove nonadherent organisms and were treated with trypsin-EDTA (0.05% trypsin, 0.5% EDTA) in PBS to release them from the plastic support. Well contents were agitated, and dilutions were plated on solid medium to yield the number of adherent bacteria per monolayer. Percent adherence was calculated by dividing the number of adherent cfu per monolayer by the number of inoculated cfu.

As depicted in Table 1 below (the Tables appear at the end of the descriptive text), this strain adhered quite efficiently, with nearly 90% of the inoculum binding to the monolayer. Adherence by the mutant expressing HMW1 but not HMW2 (HMW2⁻) was also quite

efficient and comparable to that by the wild type strain. In contrast, attachment by the strain expressing HMW2 but deficient in expression of HMW1 (HMW1$^-$) was decreased about 15-fold relative to the wild type. Adherence by the double mutant (HMW1$^-$/HMW2$^-$) was decreased even further, approximately 50-fold compared with the wild type and approximately 3-fold compared with the HMW1 mutant. Considered together, these results suggest that both the HMW1 protein and the, HMW2 protein influence attachment to Chang epithelial cells. Interestingly, optimal adherence to this cell line appears to require HMW1 but not HMW2.

<u>Example 4</u>:

This Example illustrates the preparation and expression of HMW3 and HMW4 proteins and their function as adhesins.

Using the plasmids pHMW1-16 and pHMW1-17 (see Example 3) and following a scheme similar to that employed with strain 12 as described in Example 3, three non-typeable <u>Haemophilus</u> strain 5 mutants were isolated, including one with the kanamycin gene inserted into the <u>hmw1</u>-like (designated <u>hmw3</u>) locus, a second with an insertion in the <u>hmw2</u>-like (designated <u>hmw4</u>) locus, and a third with insertions in both loci. As predicted, Western immunoblot analysis demonstrated that the mutant with insertion of the kanamycin cassette into the <u>hmw1</u>-like locus had lost expression of the HMW3 125-kD protein, while the mutant with insertion into the <u>hmw2</u>-like locus failed to express the HMW4 123-kD protein. The mutant with a double insertion was unable to express either of the high molecular weight proteins.

As shown in Table 1 below, wild type strain 5 demonstrated high level adherence, with almost 80% of the inoculum adhering per monolayer. Adherence by the mutant deficient in expression of the HMW2-like protein (i.e. HMW4 protein) was also quite high. In contrast,

adherence by the mutant unable to express the HMW1-like protein (i.e. HMW3 protein) was reduced about 5-fold relative to the wild type, and attachment by the double mutant was diminished even further (approximately 25-fold). Examination of Giemsa-stained samples confirmed these observations (not shown). Thus, the results with strain 5 for proteins HMW3 and HMW4 corroborate the findings with strain 12 and the HMW1 and HMW2 proteins.

Example 5:

This Example contains additional data concerning the adhesin properties of the HMW1 and HMW2 proteins.

To confirm an adherence function for the HMW1 and HMW2 proteins and to examine the effect of HMW1 and HMW2 independently of other *H. influenzae* surface structures, the *hmw1* and the *hmw2* gene clusters were introduced into *E. coli* DH5α, using plasmids pHMW1-14 and pHMW2-21, respectively. As a control, the cloning vector, pT7-7, was also transformed into *E. coli* DH5α. Western blot analysis demonstrated that *E. coli* DH5α containing the *hmw1* genes expressed a 125 kDa protein, while the same strain harboring the *hmw2* genes expressed a 120-kDa protein. *E. coli* DH5α containing pT7-7 failed to react with antiserum against recombinant HMW1. Transmission electron microscopy revealed no pili or other surface appendages on any of the *E. coli* strains.

Adherence by the *E. coli* strains was quantitated and compared with adherence by wild type non-typeable *H. influenzae* strain 12. As shown in Table 2 below, adherence by *E. coli* DH5α containing vector alone was less than 1% of that for strain 12. In contrast, *E. coli* DH5α harboring the *hmw1* gene cluster demonstrated adherence levels comparable to those for strain 12. Adherence by *E. coli* DH5α containing the *hmw2* genes was approximately 6-fold lower than attachment by strain 12 but was increased 20-fold over adherence by *E. coli* DH5α with pT7-7 alone. These results indicate that the HMW1

and HMW2 proteins are capable of independently mediating attachment to Chang conjunctival cells. These results are consistent with the results with the H. influenzae mutants reported in Examples 3 and 4, providing further evidence that, with Chang epithelial cells, HMW1 is a more efficient adhesin than is HMW2.

Experiments with E. coli HB101 harboring pT7-7, pHMW1-14, or pHMW2-21 confirmed the results obtained with the DH5α derivatives (see Table 2).

Example 6:

This Example illustrates the copurification of HMW1 and HMW2 proteins from wild-type non-typeable H. influenzae strain.

HMW1 and HMW2 were isolated and purified from non-typeable H. influenzae (NTHI) strain 12 in the following manner. Non-typeable Haemophilus bacteria from frozen stock culture were streaked onto a chocolate plate and grown overnight at 37°C in an incubator with 5% CO_2. 50ml starter culture of brain heart infusion (BHI) broth, supplemented with 10 μg/ml each of hemin and NAD was inoculated with growth on chocolate plate. The starter culture was grown until the optical density (O.D. - 600nm) reached 0.6 to 0.8 and then the bacteria in the starter culture was used to inoculate six 500 ml flasks of supplemented BHI using 8 to 10 ml per flask. The bacteria were grown in 500 ml flasks for an additional 5 to 6 hours at which time the O.D. was 1.5 or greater. Cultures were centrifuged at 10,000 rpm for 10 minutes.

Bacterial pellets were resuspended in a total volume of 250 ml of an extraction solution comprising 0.5 M NaCl, 0.01 M Na_2EDTA, 0.01 M Tris 50 μM 1,10-phenanthroline, pH 7.5. The cells were not sonicated or otherwise disrupted. The resuspended cells were allowed to sit on ice at 0°C for 60 minutes. The resuspended cells were centrifuged at 10,000 rpm for 10 minutes at 4°C to remove the majority of intact cells and cellular

debris. The supernatant was collected and centrifuged at 100,000 x g for 60 minutes at 4°C. The supernatant again was collected and dialyzed overnight at 4°C against 0.01 M sodium phosphate, pH 6.0.

The sample was centrifuged at 10,000 rpm for 10 minutes at 4°C to remove insoluble debris precipitated from solution during dialysis. The supernatant was applied to a 10 ml CM Sepharose column which has been pre-equilibrated with 0.01 M sodium phosphate, pH 6. Following application to this column, the column was washed with 0.01 M sodium phosphate. Proteins were elevated from the column with a 0 - 0.5M KCl gradient in 0.01 M Na phosphate, pH 6 and fractions were collected for gel examination. Coomassie gels of column fractions were carried out to identify those fractions containing high molecular weight proteins. The fractions containing high molecular weight proteins were pooled and concentrated to a 1 to 3 ml volume in preparation for application of sample to gel filtration column.

A Sepharose CL-4B gel filtration column was equilibrated with phosphate-buffered saline, pH 7.5. The concentrated high molecular weight protein sample was applied to the gel filtration column and column fractions were collected. Coomassie gels were performed on the column fractions to identify those containing high molecular weight proteins. The column fractions containing high molecular weight proteins were pooled.

<u>Example 7</u>:

This Example illustrates the use of specified HMW1 and HMW2 proteins in immunization studies.

The copurified HMW1 and HMW2 proteins prepared as described in Example 6 were tested to determine whether they would protect against experimental otitis media caused by the homologous strain.

Healthy adult chinchillas, 1 to 2 years of age with weights of 350 to 500g, received three monthly

subcutaneous injections with 40 μg of an HMW1-HMW2 protein mixture in Freund's adjuvant. Control animals received phosphate-buffered saline in Freunds' adjuvant. One month after the last injection, the animals were challenged by intrabullar inoculation with 300 cfu of NTHI strain 12.

Middle ear infection developed in 5 of 5 control animals versus 5 of 10 immunized animals. Although only 5 of 10 chinchillas were protected in this test, the test conditions are very stringent, requiring bacteria to be injected directly into the middle ear space and to proliferate in what is in essence a small abscess cavity. As seen from the additional data below, complete protection of chinchillas can be achieved.

The five HMW1/HMW2-immunized animals that did not develop otitis media demonstrated no signs of middle ear inflammation when examined by otoscopy nor were middle ear effusions detectable.

Among the five HMW1/HMW2-immunized animals that became infected, the total duration of middle ear infection as assessed by the persistence of culture-positive middle ear fluid was not different from controls. However, the degree of inflammation of the tympanic membranes was subjectively less than in the HMW1/HMW2-immunized animals. When quantitative bacterial counts were performed on the middle ear fluid specimens recovered from infected animals, notable differences were apparent between the HMW1/HMW2-immunized and PBS-immunized animals (Figure 17). Shown in Figure 17 are quantitative middle ear fluid bacterial counts from animals on day 7 post-challenge, a time point associated with the maximum colony counts in middle ear fluid. The data were log-transformed for purpose of statistical comparison. The data from the control animals are shown on the left and data from the high molecular weight protein immunized animals on the right. The two

horizontal lines indicate the respective means and standard derivations of middle ear fluid colony counts for only the infected animals in each group. As can be seen from this Figure, the HMW1/HMW2-immunized animals had significantly lower middle ear fluid bacterial counts than the PBS-immunized controls, geometric means of 7.4 X 10^6 and 1.3 X 10^5, respectively (p=0.02, Students' t-test)

Serum antibody titres following immunization were comparable in uninfected and infected animals. However, infection in immunized animals was uniformly associated with the appearance of bacteria down-regulated in expression of the HMW proteins, suggesting bacterial selection in response to immunologic pressure.

Although this data shows that protection following immunization was not complete, this data suggests the HMW adhesin proteins are potentially important protective antigens which may comprise one component of a multi-component NTHI vaccine.

In addition, complete protection has been achieved in the chinchilla model at lower dosage challenge, as set forth in Table 3 below.

Groups of five animals were immunized with 20 μg of the HMW1-HMW2 mixture prepared as described in Example 6 on days 1, 28 and 42 in the presence of alum. Blood samples were collected on day 53 to monitor the antibody response. On day 56, the left ear of animals was challenged with about 10 cfu of <u>H. influenzae</u> strain 12. Ear infection was monitored on day 4. Four animals in Group 3 were infected previously by <u>H. influenzae</u> strain 12 and were recovered completely for at least one month before the second challenge.

<u>Example 8</u>:

This Example illustrates the provision of synthetic peptides corresponding to a portion only of the HMW1 protein.

A number of synthetic peptides were derived from HMW1. Antisera then were raised to these peptides. The anti-peptide antisera to peptide HMW1-P5 was shown to recognize HMW1. Peptide HMW1-P5 covers amino acids 1453 to 1481 of HMW1, has the sequence VDEVIEAKRILEKVKDLSDEEREALAKLG (SEQ ID No: 11), and represents bases 1498 to 1576 in Figure 10.

This finding demonstrates that the DNA sequence and the derived protein is being interpreted in the correct reading frame and that peptides derived from the sequence can be produced which will be immunogenic.

Example 9:

This Example describes the generation of monoclonal antibodies to the high molecular weight proteins of non-typeable H. influenzae.

Monoclonal antibodies were generated using standard techniques. In brief, female BALB/c mice (4 to 6 weeks old) were immunized by intraperitoneal injection with high molecular weight proteins purified from nontypable Haemophilus strain 5 or strain 12, as described in Example 6. The first injection of 40 to 50 μg of protein was administered with Freund's complete adjuvant and the second dose, received four to five weeks after the first, was administered with phosphate-buffered saline. Three days following the second injection, the mice were sacrificed and splenic lymphocytes were fused with SP2/0-Ag14 plasmacytoma cells.

Two weeks following fusion, hybridoma supernatants were screened for the presence of high molecular weight protein specific antibodies by a dot-blot assay. Purified high molecular weight proteins at a concentration of 10 μg per ml in TRIS-buffered saline (TBS), were used to sensitize nitrocellulose sheets (Bio-Rad Laboratories, Richmond, CA) by soaking for 20 minutes. Following a blocking step with TBS-3% gelatin, the nitrocellulose was incubated for 60 minutes at room

temperature with individual hybridoma supernatants, at a 1:5 dilution in TBS-0. 1 % Tween, using a 96-well Bio-Dot micro-filtration apparatus (Bio-Rad). After washing, the sheets were incubated for one hour with alkaline-phosphatase-conjugated affinity isolated goat-anti(mouse IgG + IgM) antibodies (Tago, Inc., Burlingame, CA). Following additional washes, positive supernatants were identified by incubation of the nitrocellulose sheet in alkaline phosphatase buffer (0.10 M TRIS, 0.10 M NaCl, 0.005 M $MgCl_2$,) containing nitroblue tetrazolium (0.1 mg/ml) and 5-bromo-4-chloro-3-indoyl phosphate (BCIP) (0.05 mg/ml).

For the antibody isotyping and immunoelectron microscopy studies to be described below, the monoclonal antibodies were purified from hybridoma supernatants. The antibodies recovered in this work were all of the IgG class. To purify the monoclonal antibodies, the hybridoma supernatants were first subjected to ammonium sulfate precipitation (50% final concentration at 0°C). Following overnight incubation, the precipitate was recovered by centrifugation and resolubilized in phosphate buffered saline. The solution was then dialyzed overnight against 0.01 M sodium phosphate buffer, pH 6.0. The following day the sample was applied to a DEAE-Sephacel column preequilibrated with the same phosphate buffer and the proteins were subsequently eluted with a KCl gradient. Column fractions containing the monoclonal antibodies were identified by examination of samples on Coomassie gels for protein bands typical of light and heavy chains.

The isotype of each monoclonal antibody was determined by immunodiffusion using the Ouchterlony method. Immunodiffusion plates were prepared on glass slides with 10 ml of 1% DNA-grade agarose (FMC Bioproducts, Rockland, ME) in phospate-buffered saline. After the agarose solidified, 5-mm wells were punched

into the agarose in a circular pattern. The center well contained a concentrated preparation of the monoclonal antibody being evaluated and the surrounding wells contained goat anti-mouse subclass-specific antibodies (Tago). The plates were incubated for 48 hours in a humid chamber at 4°C and then examined for white lines of immunoprecipitation.

Hybridoma supernatants which were reactive in the dot-blot assay described above were examined by Western blot analysis, both to confirm the reactivity with the high molecular weight proteins of the homologous nontypable Haemophilus strain and to examine the cross-reactivity with similar proteins in heterologous strains. Nontypable Haemophilus influenzae cell sonicates containing 100 μg of total protein were solubilized in electrophoresis sample buffer, subjected to SDS-polyacrylamide gel electrophoresis on 7.5% acrylamide gels, and transferred to nitrocellulose using a Genie electrophoretic blotter (Idea Scientific Company, Corvallis, OR) for 45 min at 24 V. After transfer, the nitrocellulose sheet was blocked and then probed sequentially with the hybridoma supernatant, with alkaline phosphatase-conjugated goat-anti(mouse IgG + IgM) second antibody, and finally bound antibodies were detected by incubation with nitroblue tetrazolium/BCIP solution. This same assay was employed to examine the reactivity of the monoclonals with recombinant fusion proteins expressed in *E. coli* (see below).

In preparation for immunoelectronmicroscopy, bacteria were grown overnight on supplemented chocolate agar and several colonies were suspended in phosphate-buffered-saline containing 1 % albumin. A 20-μl drop of this bacterial suspension was then applied to a carbon-coated grid and incubated for 2 min. Excess fluid was removed and the specimen was then incubated for 5 min with the purified high molecular weight protein-specific

monoclonal antibody being analyzed. Following removal of excess liquid and a wash with phosphatebuffered saline, the specimen was incubated with anti-mouse IgG conjugated to 10-nm colloidal gold particles. Following final washes with phosphate-buffered saline, the sample was rinsed with distilled water. Staining of the bacterial cells was performed with 0.5% uranyl acetate for 1 min. Samples were then examined in a Phillips 201c electron microscope.

Fourteen different hybridomas were recovered which produced monoclonal antibodies reactive with the purified HMW1 and HMW2 proteins of nontypable Haemophilus strain 12 in the immunoblot screening assay. Of the monoclonals screened by immunoelectron microscopy to date, as described below, two were demonstrated to bind surface epitopes on prototype strain 12. These two monoclonal antibodies, designated AD6 (ATCC _______) and 10C5 (ATCC _______), were both of the IgG1 subclass.

Example 10:

This Example describes the identification of surface-exposed B-cell epitopes of high molecular weight proteins of non-typeable H. influenzae.

To map epitopes recognized by the monoclonal antibodies, their reactivity with a panel of recombinant fusion proteins expressed by pGEMEX® recombinant plasmids was examined. These plasmids were constructed by cloning various segments of the hmw1a or hmw2A structural genes into T7 expression vectors pGEMEX® -1 and GEMEX®-2 (Promega Corporation, Madison, WI). Shown in Figures 18 and 19 are the schematic diagrams depicting the segments derived from the hmw1 and hmw2 gene clusters cloned into the pGEMEX® expression plasmids. These segments were inserted such that in-frame fusions were created at each junction site. Thus, these plasmids encode recombinant fusion proteins containing pGEMEX®-encoded T7 gene 10 amino acids in the regions indicated by the hatched bars

and hmw1a or hmw2A encoded amino acids in the regions indicated by the black bars in these Figures. A stop codon is present at the junction of the black and white segments of each bar.

Four discrete sites within the hmw1A structural gene were selected as the 5′ ends of the hmw1 inserts. For each 5′ end, a series of progressively smaller inserts was created by taking advantage of convenient downstream restriction sites. The first recombinant plasmid depicted in Figure 18 was constructed by isolating a 4.9 kbp BamHI-HindIII fragment from pHMW1-14 (Example 1, Figure 5A), which contains the entire hmw1 gene cluster and inserting it into BamHI-HindIII digested pGEMEX®-1. The second recombinant plasmid in this set was constructed by digesting the "parent" plasmid with BstEII-HindIII, recovering the 6.8 kbp larger fragment, blunt-ending with Klenow DNA polymerase, and religating. The third recombinant plasmid in this set was constructed by digesting the "parent" plasmid with ClaI-HindIII, recovering the 6.0 kbp larger fragment, blunt-ending, and religating. The next set of four hmw1 recombinant plasmids was derived from a "parent" plasmid constructed by ligating a 2.2 kbp EcoRI fragment from the hmw1 gene cluster into EcoRI-digested pGEMEX®-2. The other three recombinant plasmids in this second set were constructed by digesting at downstream BstEII, EcoRV, and ClaI sites, respectively, using techniques similar to those just described. The third set of three recombinant plasmids depicted was derived from a "parent" plasmid constructed by double-digesting the first recombinant plasmid described above (i.e. the one containing the 4.9 kbp BamHI-HindIII fragment) with BamHI and ClaI, blunt-ending, and religating. This resulted in a construct encoding a recombinant protein with an in-frame fusion at the ClaI site of the hmw1A gene. The remaining two plasmids in this third set were constructed by digesting

at downstream BstEII and EcoRV sites, respectively. Finally, the fourth set of two recombinant plasmids was derived from a "parent" plasmid constructed by double-digesting the original BamHI-HindIII construct with HincII and EcoRV, then religating. This resulted in a construct encoding a recombinant protein with an in-frame fusion at the EcoRV site of the hmw1A gene. The remaining plasmid in this fourth set was constructed by digesting at the downstream BstEII site.

Three discrete sites with the hmw2A structural gene were selected as the 5′ ends of the hmw2 inserts. The first recombinant plasmid depicted in Figure 19 was constructed by isolating a 6.0 kbp EcoRI-XhoI fragment from pHMW2-21, which contains the entire hmw2 gene cluster, and inserting it into EcoRI-SalI digested pGEMEX®-1. The second recombinant plasmid in this set was constructed by digesting at an MluI site near the 3′ end of the hmw2A gene. The second set of two hmw2 recombinant plasmids was derived from a "parent" plasmid constructed by isolating a 2.3 kbp HindIII fragment from pHMW2-21 and inserting it into HindIII-digested pGEMEX®-2. The remaining plasmid in this second set was constructed by digesting at the downstream MluI site. Finally, the last plasmid depicted was constructed by isolating a 1.2 kbp HincII-HindIII fragment from the indicated location in the hmw2 gene cluster and inserting it into HincII-HindIII digested pGEMEX®-1.

Each of the recombinant plasmids was used to transform E. coli strain JM101. The resulting transformants were used to generate the recombinant fusion proteins employed in the mapping studies. To prepare recombinant proteins, the transformed E. coli strains were grown to an A_{600} of 0.5 in L broth containing 50 μg of ampicillin per ml. IPTG was then added to 1mM and mGP1-2, the M13 phage containing the T7 RNA polymerase gene, was added at multiplicity of infection

of 10. One hour later, cells were harvested, and a sonicate of the cells was prepared. The protein concentrations of the samples were determined and cell sonicates containing 100 μg of total protein were solubilized in electrophoresis sample buffer, subjected to SDS-polyacrylamide gel electrophoresis, and examined on Coomassie gels to assess the expression level of recombinant fusion proteins. Once high levels of expression of the recombinant fusion proteins were confirmed, the cell sonicates were used in the Western blot analyses described above.

Shown in Figure 20 is an electron micrograph demonstrating surface binding of Mab AD6 to representative nontypable Haemophilus influenzae strains. In the upper left panel of the Figure is nontypable Haemophilus strain 12 and in the upper right panel is a strain 12 derivative which no longer expressed the high molecular weight proteins. As can be seen, colloidal gold particles decorate the surface of strain 12, indicating bound AD6 antibody on the surface. In contrast, no gold particles are evident on the surface of the strain 12 mutant which no longer expresses the high molecular weight proteins. These results indicate that monoclonal antibody AD6 is recognizing a surface-exposed epitope on the high molecular weight proteins of strain 12. Analogous studies were performed with monoclonal antibody 10C5 demonstrating it too bound to surface-accessible epitopes on the high molecular weight HMW1 and HMW2 proteins of strain 12.

Having identified two surface-binding monoclonals, the epitope which each monoclonal recognized was mapped. To accomplish this task, the two sets of recombinant plasmids containing various portions of either the hmw1a or hmw2A structural genes (Figures 18 and 19) were employed. With these complementary sets of recombinant plasmids, the epitopes recognized by the monoclonal

antibodies were mapped to relatively small regions of the very large HMW1 and HMW2 proteins.

To localize epitopes recognized by Mab AD6, the pattern of reactivity of this monoclonal antibody with a large set of recombinant fusion protein was examined. Figure 21 is a Western blot which demonstrates the pattern of reactivity of Mab AD6 with five recombinant fusion proteins, a relevant subset of the larger number originally examined. From analysis of the pattern of reactivity of Mab AD6 with this set of proteins, one is able to map the epitope it recognizes to a very short segment of the HMW1 and HMW2 proteins. A brief summary of this analysis follows. For reference, the relevant portions of the <u>hmw1A</u> or <u>hmw2A</u> structural genes which were expressed in the recombinant proteins being examined are indicated in the diagram at the top of the figure. As shown in lane 1, Mab AD6 recognizes an epitope encoded by fragment 1, a fragment which encompasses the distal one-fourth of the <u>hmw1A</u> gene. Reactivity is lost when only the portion of the gene comprising fragment 2 is expressed. This observation localizes the AD6 epitope somewhere within the last 180 amino acids at the carboxy-terminal end of the HMW1 protein. Mab AD6 also recognizes an epitope encoded by fragment 3, derived from the <u>hmw2A</u> structural gene. This is a rather large fragment which encompasses nearly one-third of the gene. Reactivity is lost when fragment 4 is expressed. The only difference between fragments 3 and 4 is that the last 225 base pairs at the 3′ end of the <u>hmw2A</u> structural gene were deleted in the latter construct. This observation indicates that the AD6 epitope is encoded by this short terminal segment of the <u>hmw2A</u> gene. Strong support for this idea is provided by the demonstrated binding of Mab AD6 to the recombinant protein encoded by fragment 5, a fragment encompassing the distal one-tenth of the <u>hmw2A</u> structural gene. Taken together, these data

identify the AD6 epitope as common to both the HMW1 and HMW2 proteins and place its location with 75 amino acids of the carboxy termini of the two proteins.

Figure 22 is a Western blot demonstrating the pattern of reactivity of Mab 10C5 with the same five recombinant fusion proteins examined in Figure 21. As shown in lane 1, Mab 10C5 recognizes an epitope encoded by fragment 1. In contrast to Mab AD6, Mab 10C5 also recognizes an epitope encoded by fragment 2. Also in contrast to Mab AD6, Mab 10C5 does not recognize any of the hmw2A-derived recombinant fusion proteins. Thus, these data identify the 10C5 epitope as being unique to the HMW1 protein and as being encoded by the fragment designated as fragment 2 in this figure. This fragment corresponds to a 155-amino acid segment encoded by the EcoRV-BstEII segment of the hmw1A structural gene.

Having identified the approximate locations of the epitopes on HMW1 and HMW2 recognized by the two monoclonals, the extent to which these epitopes were shared by the high molecular weight proteins of heterologous nontypable Haemophilus strains was next determined. When examined in Western blot assays with bacterial cell sonicates, Mab AD6 was reactive with epitopes expressed on the high molecular weight proteins of 75% of the inventor's collection of more than 125 nontypable Haemophilus influenzae strains. In fact, this monoclonal appeared to recognize epitopes expressed on high molecular weight proteins in virtually all nontypable Haemophilus strains which we previously identified as expressing HMW1/HMW2-like proteins. Figure 23 is an example of a Western blot demonstrating the reactivity of Mab AD6 with a representative panel of such heterologous strains. As can be seen, the monoclonal antibody recognizes one or two bands in the 100 to 150 kDa range in each of these strains. For reference, the strain shown in lane 1 is prototype strain 12 and the two

bands visualized represent HMW1 and HMW2 as the upper and lower immunoreactive bands, respectively.

In contrast to the broad cross-reactivity observed with Mab AD6, Mab 10C5 was much more limited in its ability to recognize high molecular weight proteins in heterologous strains. Mab 10C5 recognized high molecular weight proteins in approximately 40% of the strains which expressed HMW1/HMW2-like proteins. As was the case with Mab AD6, Mab 10C5 did not recognize proteins in any the nontypable Haemophilus strains which did not express HMW1/HMW2-like proteins.

In a limited fashion, the reactivity of Mab AD6 with surface-exposed epitopes on the heterologous strains has been examined. In the bottom two panels of Figure 20 are electron micrographs demonstrating the reactivity of Mab AD6 with surface-accessible epitopes on nontypable Haemophilus strains 5 and 15. As can be seen, abundant colloidal-gold particles are evident on the surfaces of each of these strains, confirming their surface expression of the AD6 epitope. Although limited in scope, these data suggest that the AD6 epitope may be a common surface-accessible epitope on the high molecular weight adhesion proteins of most nontypable *Haemophilus influenzae* which express HMW1/HMW2-like proteins.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides high molecular weight proteins of non-typeable Haemophilus, genes coding for the same and vaccines incorporating such proteins. Modifications are possible within the scope of this invention.

TABLE 1: Effect of mutation of high molecular weight proteins on adherence to Chang epithelial cells by nontypable *H. influenzae*.

	ADHERENCE % *	
Strain	**% Inoculation**	**Relative to wild Type†**
Strain 12 derivatives wild type	87.76 ± 5.9	100.0 ± 6.7
HMW1⁻ mutant	6.0 ± 0.9	6.8 ± 1.0
HMW2⁻ mutant	89.9 ± 10.8	102.5 ± 12.3
HMW1⁻/HMW2⁻ mutant	2.0 ± 0.3	2.3 ± 0.3
Strain 5 derivatives wild type	78.7 ± 3.2	100.0 ± 4.1
HMW1-like mutant	15.7 ± 2.6	19.9 ± 3.3
HMW2-like mutant	103.7 ± 14.0	131.7 ± 17.8
double mutant	3.5 ± 0.6	4.4 ± 0.8

* Numbers represent mean (± standard error of the mean) of measurements in triplicate or quadruplicate from representative experiments.

† Adherence values for strain 12 derivatives are relative to strain 12 wild type; values for strain 5 derivatives are relative to strain 5 wild type.

TABLE 2: Adherence by *E. coli* DH5α and HB101 harboring *hmw1* or *hmw2* gene clusters.

Strain*	Adherence relative to *H. influenzae* strain 12†
DH5α (pT7-7)	0.7 ± 0.02
DH5α (pHMW1-14)	114.2 ± 15.9
DH5α (pHMW2-21)	14.0 ± 3.7
HB101 (pT7-7)	1.2 ± 0.5
HB101 (pHMW1-14)	93.6 ± 15.8
HB101 (pHMW2-21)	3.6 ± 0.9

* The plasmid pHMW1-14 contains the *hmw1* gene cluster, while pHMW2-21 contains the *hmw2* gene cluster; pT7-7 is the cloning vector used in these constructs.

† Numbers represent the mean (± standard error of the mean) of measurements made in triplicate from representative experiments.

TABLE 3: Protective ability of HMW protein against non-typeable *H. influenzae* challenge in chinchilla model

Group (#)	Antigens	Total Animals	Number of Animals Showed Positive Ear Infection		
			Tympano-gram	Otosco-pic Examin-ation	cfu of Bacteria /10 μL
1	HMW	5	0	0	0
2	None	5	5	5	850-3200 (4/5)
3	Convalescent	4	0	0	0

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Barenkamp, Stephen J

(ii) TITLE OF INVENTION: High Molecular Weight Surface Proteins of Non-Typeable Haemophilus

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Shoemaker and Mattare, Ltd.
(B) STREET: 2001 Jefferson Davis Hwy., 1203 Crystal Plaza Bldg. 1
(C) CITY: Arlington
(D) STATE: Virginia
(E) COUNTRY: U.S.A.
(F) ZIP: 22202-0286

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

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(C) CLASSIFICATION:

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(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Berkstresser, Jerry W
(B) REGISTRATION NUMBER: 22,651
(C) REFERENCE/DOCKET NUMBER: 1038-557

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (703) 415-0810
(B) TELEFAX: (703) 415-0813

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5116 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ACTTAGCGTT AAAGCCACTT TCCGCTATGT TACTATCTTT AGGTGTAACA TCTATTCCAC 540
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ACAGGTTATT ATTATG 5116

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1536 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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1 5 10 15

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Gly Ser Glu Lys Pro Ala Arg Met Lys Val Arg His Leu Ala Leu Lys
35 40 45

Pro Leu Ser Ala Met Leu Leu Ser Leu Gly Val Thr Ser Ile Pro Gln
50 55 60

Ser Val Leu Ala Ser Gly Leu Gln Gly Met Asp Val Val His Gly Thr
65 70 75 80

Ala Thr Met Gln Val Asp Gly Asn Lys Thr Ile Ile Arg Asn Ser Val
85 90 95

Asp Ala Ile Ile Asn Trp Lys Gln Phe Asn Ile Asp Gln Asn Glu Met
100 105 110

Val Gln Phe Leu Gln Glu Asn Asn Asn Ser Ala Val Phe Asn Arg Val
115 120 125

Thr Ser Asn Gln Ile Ser Gln Leu Lys Gly Ile Leu Asp Ser Asn Gly
130 135 140

Gln Val Phe Leu Ile Asn Pro Asn Gly Ile Thr Ile Gly Lys Asp Ala
145 150 155 160

Ile Ile Asn Thr Asn Gly Phe Thr Ala Ser Thr Leu Asp Ile Ser Asn
165 170 175

Glu Asn Ile Lys Ala Arg Asn Phe Thr Phe Glu Gln Thr Lys Asp Lys
180 185 190

Ala Leu Ala Glu Ile Val Asn His Gly Leu Ile Thr Val Gly Lys Asp
195 200 205

Gly Ser Val Asn Leu Ile Gly Gly Lys Val Lys Asn Glu Gly Val Ile
210 215 220

Ser Val Asn Gly Gly Ser Ile Ser Leu Leu Ala Gly Gln Lys Ile Thr
225 230 235 240

Ile Ser Asp Ile Ile Asn Pro Thr Ile Thr Tyr Ser Ile Ala Ala Pro
245 250 255

Glu Asn Glu Ala Val Asn Leu Gly Asp Ile Phe Ala Lys Gly Gly Asn
260 265 270

Ile Asn Val Arg Ala Ala Thr Ile Arg Asn Gln Gly Lys Leu Ser Ala
275 280 285

Asp Ser Val Ser Lys Asp Lys Ser Gly Asn Ile Val Leu Ser Ala Lys
290 295 300

Glu Gly Glu Ala Glu Ile Gly Gly Val Ile Ser Ala Gln Asn Gln Gln
305 310 315 320

Ala Lys Gly Gly Lys Leu Met Ile Thr Gly Asp Lys Val Thr Leu Lys
325 330 335

Thr Gly Ala Val Ile Asp Leu Ser Gly Lys Glu Gly Gly Glu Thr Tyr
340 345 350

Leu Gly Gly Asp Glu Arg Gly Glu Gly Lys Asn Gly Ile Gln Leu Ala
355 360 365

Lys Lys Thr Ser Leu Glu Lys Gly Ser Thr Ile Asn Val Ser Gly Lys
370 375 380

Glu Lys Gly Gly Arg Ala Ile Val Trp Gly Asp Ile Ala Leu Ile Asp
385 390 395 400

Gly Asn Ile Asn Ala Gln Gly Ser Gly Asp Ile Ala Lys Thr Gly Gly
405 410 415

Phe Val Glu Thr Ser Gly His Asp Leu Phe Ile Lys Asp Asn Ala Ile
420 425 430

Val Asp Ala Lys Glu Trp Leu Leu Asp Phe Asp Asn Val Ser Ile Asn
435 440 445

Ala Glu Thr Ala Gly Arg Ser Asn Thr Ser Glu Asp Asp Glu Tyr Thr
450 455 460

Gly Ser Gly Asn Ser Ala Ser Thr Pro Lys Arg Asn Lys Glu Lys Thr
465 470 475 480

Thr Leu Thr Asn Thr Thr Leu Glu Ser Ile Leu Lys Lys Gly Thr Phe
485 490 495

Val Asn Ile Thr Ala Asn Gln Arg Ile Tyr Val Asn Ser Ser Ile Asn
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Leu Ser Asn Gly Ser Leu Thr Leu Trp Ser Glu Gly Arg Ser Gly Gly
515 520 525

Gly Val Glu Ile Asn Asn Asp Ile Thr Thr Gly Asp Asp Thr Arg Gly
530 535 540

Ala Asn Leu Thr Ile Tyr Ser Gly Gly Trp Val Asp Val His Lys Asn
545 550 555 560

Ile Ser Leu Gly Ala Gln Gly Asn Ile Asn Ile Thr Ala Lys Gln Asp
565 570 575

Ile Ala Phe Glu Lys Gly Ser Asn Gln Val Ile Thr Gly Gln Gly Thr
580 585 590

Ile Thr Ser Gly Asn Gln Lys Gly Phe Arg Phe Asn Asn Val Ser Leu
595 600 605

Asn Gly Thr Gly Ser Gly Leu Gln Phe Thr Thr Lys Arg Thr Asn Lys
610 615 620

Tyr Ala Ile Thr Asn Lys Phe Glu Gly Thr Leu Asn Ile Ser Gly Lys
625 630 635 640

Val Asn Ile Ser Met Val Leu Pro Lys Asn Glu Ser Gly Tyr Asp Lys
645 650 655

Phe Lys Gly Arg Thr Tyr Trp Asn Leu Thr Ser Leu Asn Val Ser Glu
660 665 670

Ser Gly Glu Phe Asn Leu Thr Ile Asp Ser Arg Gly Ser Asp Ser Ala
675 680 685

Gly Thr Leu Thr Gln Pro Tyr Asn Leu Asn Gly Ile Ser Phe Asn Lys
690 695 700

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Lys Ala Pro Ile Gly Ile Asn Lys Tyr Ser Ser Leu Asn Tyr Ala Ser
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900 905 910

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930 935 940

Lys Asn Leu Ser Ile Thr Thr Asn Ser Ser Ser Thr Tyr Arg Thr Ile
945 950 955 960

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980 985 990

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995 1000 1005

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1105 1110 1115 1120

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1235 1240 1245

Ser Leu Thr Thr Gln Ser Asn Ser Lys Ile Lys Ala Thr Thr Gly Glu
1250 1255 1260

Ala Asn Val Thr Ser Ala Thr Gly Thr Ile Gly Gly Thr Ile Ser Gly
1265 1270 1275 1280

Asn Thr Val Asn Val Thr Ala Asn Ala Gly Asp Leu Thr Val Gly Asn
1285 1290 1295

Gly Ala Glu Ile Asn Ala Thr Glu Gly Ala Ala Thr Leu Thr Thr Ser
1300 1305 1310

Ser Gly Lys Leu Thr Thr Glu Ala Ser Ser His Ile Thr Ser Ala Lys
1315 1320 1325

Gly Gln Val Asn Leu Ser Ala Gln Asp Gly Ser Val Ala Gly Ser Ile
1330 1335 1340

Asn Ala Ala Asn Val Thr Leu Asn Thr Thr Gly Thr Leu Thr Thr Val
1345 1350 1355 1360

Lys Gly Ser Asn Ile Asn Ala Thr Ser Gly Thr Leu Val Ile Asn Ala
1365 1370 1375

Lys Asp Ala Glu Leu Asn Gly Ala Ala Leu Gly Asn His Thr Val Val
1380 1385 1390

Asn Ala Thr Asn Ala Asn Gly Ser Gly Ser Val Ile Ala Thr Thr Ser
1395 1400 1405

Ser Arg Val Asn Ile Thr Gly Asp Leu Ile Thr Ile Asn Gly Leu Asn
1410 1415 1420

Ile Ile Ser Lys Asn Gly Ile Asn Thr Val Leu Leu Lys Gly Val Lys
1425 1430 1435 1440

Ile Asp Val Lys Tyr Ile Gln Pro Gly Ile Ala Ser Val Asp Glu Val
1445 1450 1455

Ile Glu Ala Lys Arg Ile Leu Glu Lys Val Lys Asp Leu Ser Asp Glu
1460 1465 1470

Glu Arg Glu Ala Leu Ala Lys Leu Gly Val Ser Ala Val Arg Phe Ile
1475 1480 1485

Glu Pro Asn Asn Thr Ile Thr Val Asp Thr Gln Asn Glu Phe Ala Thr
1490 1495 1500

Arg Pro Leu Ser Arg Ile Val Ile Ser Glu Gly Arg Ala Cys Phe Ser
1505 1510 1515 1520

Asn Ser Asp Gly Ala Thr Val Cys Val Asn Ile Ala Asp Asn Gly Arg
1525 1530 1535

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4937 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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TAAATATACA AGATAATAAA AATAAATCAA GATTTTTGTG ATGACAAACA ACAATTACAA      60
CACCTTTTTT GCAGTCTATA TGCAAATATT TTAAAAAAAT AGTATAAATC CGCCATATAA     120
AATGGTATAA TCTTTCATCT TTCATCTTTA ATCTTTCATC TTTCATCTTT CATCTTTCAT     180
CTTTCATCTT TCATCTTTCA TCTTTCATCT TTCATCTTTC ATCTTTCATC TTTCATCTTT     240
CACATGAAAT GATGAACCGA GGGAAGGGAG GGAGGGGCAA GAATGAAGAG GGAGCTGAAC     300
GAACGCAAAT GATAAAGTAA TTTAATTGTT CAACTAACCT TAGGAGAAAA TATGAACAAG     360
ATATATCGTC TCAAATTCAG CAAACGCCTG AATGCTTTGG TTGCTGTGTC TGAATTGGCA     420
CGGGGTTGTG ACCATTCCAC AGAAAAAGGC TTCCGCTATG TTACTATCTT TAGGTGTAAC     480
CACTTAGCGT TAAAGCCACT TTCCGCTATG TTACTATCTT TAGGTGTAAC ATCTATTCCA     540
CAATCTGTTT TAGCAAGCGG CTTACAAGGA ATGGATGTAG TACACGGCAC AGCCACTATG     600
CAAGTAGATG GTAATAAAAC CATTATCCGC AACAGTGTTG ACGCTATCAT TAATTGGAAA     660
CAATTTAACA TCGACCAAAA TGAAATGGTG CAGTTTTTAC AAGAAAACAA CAACTCCGCC     720
GTATTCAACC GTGTTACATC TAACCAAATC TCCCAATTAA AAGGGATTTT AGATTCTAAC     780
GGACAAGTCT TTTTAATCAA CCCAAATGGT ATCACAATAG GTAAAGACGC AATTATTAAC     840
ACTAATGGCT TTACGGCTTC TACGCTAGAC ATTTCTAACG AAAACATCAA GGCGCGTAAT     900
TTCACCTTCG AGCAAACCAA AGATAAAGCG CTCGCTGAAA TTGTGAATCA CGGTTTAATT     960
ACTGTCGGTA AAGACGGCAG TGTAAATCTT ATTGGTGGCA AAGTGAAAAA CGAGGGTGTG    1020
ATTAGCGTAA ATGGTGGCAG CATTTCTTTA CTCGCAGGGC AAAAAATCAC CATCAGCGAT    1080
ATAATAAACC CAACCATTAC TTACAGCATT GCCGCGCCTG AAAATGAAGC GGTCAATCTG    1140
GGCGATATTT TTGCCAAAGG CGGTAACATT AATGTCCGTG CTGCCACTAT TCGAAACCAA    1200
GGTAAACTTT CTGCTGATTC TGTAAGCAAA GATAAAAGCG GCAATATTGT TCTTTCCGCC    1260
AAAGAGGGTG AAGCGGAAAT TGGCGGTGTA ATTTCCGCTC AAAATCAGCA AGCTAAAGGC    1320
GGCAAGCTGA TGATTACAGG CGATAAAGTC ACATTAAAAA CAGGTGCAGT TATCGACCTT    1380
TCAGGTAAAG AAGGGGGAGA AACTTACCTT GGCGGTGACG AGCGCGGCGA AGGTAAAAAC    1440
GGCATTCAAT TAGCAAAGAA AACCTCTTTA GAAAAAGGCT CAACCATCAA TGTATCAGGC    1500
AAAGAAAAAG GCGGACGCGC TATTGTGTGG GGCGATATTG CGTTAATTGA CGGCAATATT    1560
AACGCTCAAG GTAGTGGTGA TATCGCTAAA ACCGGTGGTT TTGTGGAGAC ATCGGGGCAT    1620
TATTTATCCA TTGACAGCAA TGCAATTGTT AAAACAAAAG AGTGGTTGCT AGACCCTGAT    1680
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GATGTAACAA TTGAAGCCGA AGACCCCCTT CGCAATAATA CCGGTATAAA TGATGAATTC 1740
CCAACAGGCA CCGGTGAAGC AAGCGACCCT AAAAAAAATA GCGAACTCAA AACAACGCTA 1800
ACCAATACAA CTATTTCAAA TTATCTGAAA AACGCCTGGA CAATGAATAT AACGGCATCA 1860
AGAAAACTTA CCGTTAATAG CTCAATCAAC ATCGGAAGCA ACTCCCACTT AATTCTCCAT 1920
AGTAAAGGTC AGCGTGGCGG AGGCGTTCAG ATTGATGGAG ATATTACTTC TAAAGGCGGA 1980
AATTTAACCA TTTATTCTGG CGGATGGGTT GATGTTCATA AAAATATTAC GCTTGATCAG 2040
GGTTTTTTAA ATATTACCGC CGCTTCCGTA GCTTTTGAAG GTGGAAATAA CAAAGCACGC 2100
GACGCGGCAA ATGCTAAAAT TGTCGCCCAG GGCACTGTAA CCATTACAGG AGAGGGAAAA 2160
GATTTCAGGG CTAACAACGT ATCTTTAAAC GGAACGGGTA AAGGTCTGAA TATCATTTCA 2220
TCAGTGAATA ATTTAACCCA CAATCTTAGT GGCACAATTA ACATATCTGG GAATATAACA 2280
ATTAACCAAA CTACGAGAAA GAACACCTCG TATTGGCAAA CCAGCCATGA TTCGCACTGG 2340
AACGTCAGTG CTCTTAATCT AGAGACAGGC GCAAATTTTA CCTTTATTAA ATACATTTCA 2400
AGCAATAGCA AAGGCTTAAC AACACAGTAT AGAAGCTCTG CAGGGGTGAA TTTTAACGGC 2460
GTAAATGGCA ACATGTCATT CAATCTCAAA GAAGGAGCGA AAGTTAATTT CAAATTAAAA 2520
CCAAACGAGA ACATGAACAC AAGCAAACCT TTACCAATTC GGTTTTTAGC CAATATCACA 2580
GCCACTGGTG GGGGCTCTGT TTTTTTTGAT ATATATGCCA ACCATTCTGG CAGAGGGGCT 2640
GAGTTAAAAA TGAGTGAAAT TAATATCTCT AACGGCGCTA ATTTTACCTT AAATTCCCAT 2700
GTTCGCGGCG ATGACGCTTT TAAAATCAAC AAAGACTTAA CCATAAATGC AACCAATTCA 2760
AATTTCAGCC TCAGACAGAC GAAAGATGAT TTTTATGACG GGTACGCACG CAATGCCATC 2820
AATTCAACCT ACAACATATC CATTCTGGGC GGTAATGTCA CCCTTGGTGG ACAAAACTCA 2880
AGCAGCAGCA TTACGGGGAA TATTACTATC GAGAAAGCAG CAAATGTTAC GCTAGAAGCC 2940
AATAACGCCC CTAATCAGCA AAACATAAGG GATAGAGTTA TAAAACTTGG CAGCTTGCTC 3000
GTTAATGGGA GTTTAAGTTT AACTGGCGAA AATGCAGATA TTAAAGGCAA TCTCACTATT 3060
TCAGAAAGCG CCACTTTTAA AGGAAAGACT AGAGATACCC TAAATATCAC CGGCAATTTT 3120
ACCAATAATG GCACTGCCGA AATTAATATA ACACAAGGAG TGGTAAAACT TGGCAATGTT 3180
ACCAATGATG GTGATTTAAA CATTACCACT CACGCTAAAC GCAACCAAAG AAGCATCATC 3240
GGCGGAGATA TAATCAACAA AAAAGGAAGC TTAAATATTA CAGACAGTAA TAATGATGCT 3300
GAAATCCAAA TTGGCGGCAA TATCTCGCAA AAAGAAGGCA ACCTCACGAT TTCTTCCGAT 3360
AAAATTAATA TCACCAAACA GATAACAATC AAAAAGGGTA TTGATGGAGA GGACTCTAGT 3420
TCAGATGCGA CAAGTAATGC CAACCTAACT ATTAAAACCA AAGAATTGAA ATTGACAGAA 3480
GACCTAAGTA TTTCAGGTTT CAATAAAGCA GAGATTACAG CCAAAGATGG TAGAGATTTA 3540
ACTATTGGCA ACAGTAATGA CGGTAACAGC GGTGCCGAAG CCAAAACAGT AACTTTTAAC 3600
AATGTTAAAG ATTCAAAAAT CTCTGCTGAC GGTCACAATG TGACACTAAA TAGCAAAGTG 3660
AAAACATCTA GCAGCAATGG CGGACGTGAA AGCAATAGCG ACAACGATAC CGGCTTAACT 3720

ATTACTGCAA AAAATGTAGA AGTAAACAAA GATATTACTT CTCTCAAAAC AGTAAATATC 3780

ACCGCGTCGG AAAAGGTTAC CACCACAGCA GGCTCGACCA TTAACGCAAC AAATGGCAAA 3840

GCAAGTATTA CAACCAAAAC AGGTGATATC AGCGGTACGA TTTCCGGTAA CACGGTAAGT 3900

GTTAGCGCGA CTGGTGATTT AACCACTAAA TCCGGCTCAA AAATTGAAGC GAAATCGGGT 3960

GAGGCTAATG TAACAAGTGC AACAGGTACA ATTGGCGGTA CAATTTCCGG TAATACGGTA 4020

AATGTTACGG CAAACGCTGG CGATTTAACA GTTGGGAATG GCGCAGAAAT TAATGCGACA 4080

GAAGGAGCTG CAACCTTAAC CGCAACAGGG AATACCTTGA CTACTGAAGC CGGTTCTAGC 4140

ATCACTTCAA CTAAGGGTCA GGTAGACCTC TTGGCTCAGA ATGGTAGCAT CGCAGGAAGC 4200

ATTAATGCTG CTAATGTGAC ATTAAATACT ACAGGCACCT TAACCACCGT GGCAGGCTCG 4260

GATATTAAAG CAACCAGCGG CACCTTGGTT ATTAACGCAA AAGATGCTAA GCTAAATGGT 4320

GATGCATCAG GTGATAGTAC AGAAGTGAAT GCAGTCAACG CAAGCGGCTC TGGTAGTGTG 4380

ACTGCGGCAA CCTCAAGCAG TGTGAATATC ACTGGGGATT TAAACACAGT AAATGGGTTA 4440

AATATCATTT CGAAAGATGG TAGAAACACT GTGCGCTTAA GAGGCAAGGA AATTGAGGTG 4500

AAATATATCC AGCCAGGTGT AGCAAGTGTA GAAGAAGTAA TTGAAGCGAA ACGCGTCCTT 4560

GAAAAAGTAA AAGATTTATC TGATGAAGAA AGAGAAACAT TAGCTAAACT TGGTGTAAGT 4620

GCTGTACGTT TTGTTGAGCC AAATAATACA ATTACAGTCA ATACACAAAA TGAATTTACA 4680

ACCAGACCGT CAAGTCAAGT GATAATTTCT GAAGGTAAGG CGTGTTTCTC AAGTGGTAAT 4740

GGCGCACGAG TATGTACCAA TGTTGCTGAC GATGGACAGC CGTAGTCAGT AATTGACAAG 4800

GTAGATTTCA TCCTGCAATG AAGTCATTTT ATTTTCGTAT TATTTACTGT GTGGGTTAAA 4860

GTTCAGTACG GGCTTTACCC ATCTTGTAAA AAATTACGGA GAATACAATA AAGTATTTTT 4920

AACAGGTTAT TATTATG 4937

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1477 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asn Lys Ile Tyr Arg Leu Lys Phe Ser Lys Arg Leu Asn Ala Leu
1 5 10 15

Val Ala Val Ser Glu Leu Ala Arg Gly Cys Asp His Ser Thr Glu Lys
20 25 30

Gly Ser Glu Lys Pro Ala Arg Met Lys Val Arg His Leu Ala Leu Lys
35 40 45

Pro Leu Ser Ala Met Leu Leu Ser Leu Gly Val Thr Ser Ile Pro Gln
50 55 60

Ser Val Leu Ala Ser Gly Leu Gln Gly Met Asp Val Val His Gly Thr
65 70 75 80

Ala Thr Met Gln Val Asp Gly Asn Lys Thr Ile Ile Arg Asn Ser Val
85 90 95

Asp Ala Ile Ile Asn Trp Lys Gln Phe Asn Ile Asp Gln Asn Glu Met
100 105 110

Val Gln Phe Leu Gln Glu Asn Asn Asn Ser Ala Val Phe Asn Arg Val
115 120 125

Thr Ser Asn Gln Ile Ser Gln Leu Lys Gly Ile Leu Asp Ser Asn Gly
130 135 140

Gln Val Phe Leu Ile Asn Pro Asn Gly Ile Thr Ile Gly Lys Asp Ala
145 150 155 160

Ile Ile Asn Thr Asn Gly Phe Thr Ala Ser Thr Leu Asp Ile Ser Asn
165 170 175

Glu Asn Ile Lys Ala Arg Asn Phe Thr Phe Glu Gln Thr Lys Asp Lys
180 185 190

Ala Leu Ala Glu Ile Val Asn His Gly Leu Ile Thr Val Gly Lys Asp
195 200 205

Gly Ser Val Asn Leu Ile Gly Gly Lys Val Lys Asn Glu Gly Val Ile
210 215 220

Ser Val Asn Gly Gly Ser Ile Ser Leu Leu Ala Gly Gln Lys Ile Thr
225 230 235 240

Ile Ser Asp Ile Ile Asn Pro Thr Ile Thr Tyr Ser Ile Ala Ala Pro
245 250 255

Glu Asn Glu Ala Val Asn Leu Gly Asp Ile Phe Ala Lys Gly Gly Asn
260 265 270

Ile Asn Val Arg Ala Ala Thr Ile Arg Asn Gln Gly Lys Leu Ser Ala
275 280 285

Asp Ser Val Ser Lys Asp Lys Ser Gly Asn Ile Val Leu Ser Ala Lys
290 295 300

Glu Gly Glu Ala Glu Ile Gly Gly Val Ile Ser Ala Gln Asn Gln Gln
305 310 315 320

Ala Lys Gly Gly Lys Leu Met Ile Thr Gly Asp Lys Val Thr Leu Lys
325 330 335

Thr Gly Ala Val Ile Asp Leu Ser Gly Lys Glu Gly Gly Glu Thr Tyr
340 345 350

Leu Gly Gly Asp Glu Arg Gly Glu Gly Lys Asn Gly Ile Gln Leu Ala
355 360 365

Lys Lys Thr Ser Leu Glu Lys Gly Ser Thr Ile Asn Val Ser Gly Lys
370 375 380

Glu Lys Gly Gly Phe Ala Ile Val Trp Gly Asp Ile Ala Leu Ile Asp
385 390 395 400

Gly Asn Ile Asn Ala Gln Gly Ser Gly Asp Ile Ala Lys Thr Gly Gly
405 410 415

Phe Val Glu Thr Ser Gly His Asp Leu Phe Ile Lys Asp Asn Ala Ile
420 425 430

Val Asp Ala Lys Glu Trp Leu Leu Asp Phe Asp Asn Val Ser Ile Asn
435 440 445

Ala Glu Asp Pro Leu Phe Asn Asn Thr Gly Ile Asn Asp Glu Phe Pro
450 455 460

Thr Gly Thr Gly Glu Ala Ser Asp Pro Lys Lys Asn Ser Glu Leu Lys
465 470 475 480

Thr Thr Leu Thr Asn Thr Thr Ile Ser Asn Tyr Leu Lys Asn Ala Trp
485 490 495

Thr Met Asn Ile Thr Ala Ser Arg Lys Leu Thr Val Asn Ser Ser Ile
500 505 510

Asn Ile Gly Ser Asn Ser His Leu Ile Leu His Ser Lys Gly Gln Arg
515 520 525

Gly Gly Gly Val Gln Ile Asp Gly Asp Ile Thr Ser Lys Gly Gly Asn
530 535 540

Leu Thr Ile Tyr Ser Gly Gly Trp Val Asp Val His Lys Asn Ile Thr
545 550 555 560

Leu Asp Gln Gly Phe Leu Asn Ile Thr Ala Ala Ser Val Ala Phe Glu
565 570 575

Gly Gly Asn Asn Lys Ala Arg Asp Ala Ala Asn Ala Lys Ile Val Ala
580 585 590

Gln Gly Thr Val Thr Ile Thr Gly Glu Gly Lys Asp Phe Arg Ala Asn
595 600 605

Asn Val Ser Leu Asn Gly Thr Gly Lys Gly Leu Asn Ile Ile Ser Ser
610 615 620

Val Asn Asn Leu Thr His Asn Leu Ser Gly Thr Ile Asn Ile Ser Gly
625 630 635 640

Asn Ile Thr Ile Asn Gln Thr Thr Arg Lys Asn Thr Ser Tyr Trp Gln
645 650 655

Thr Ser His Asp Ser His Trp Asn Val Ser Ala Leu Asn Leu Glu Thr
660 665 670

Gly Ala Asn Phe Thr Phe Ile Lys Tyr Ile Ser Ser Asn Ser Lys Gly
675 680 685

Leu Thr Thr Gln Tyr Arg Ser Ser Ala Gly Val Asn Phe Asn Gly Val
690 695 700

Asn Gly Asn Met Ser Phe Asn Leu Lys Glu Gly Ala Lys Val Asn Phe
705 710 715 720

Lys Leu Lys Pro Asn Glu Asn Met Asn Thr Ser Lys Pro Leu Pro Ile
725 730 735

Arg Phe Leu Ala Asn Ile Thr Ala Thr Gly Gly Gly Ser Val Phe Phe
740 745 750

Asp Ile Tyr Ala Asn His Ser Gly Arg Gly Ala Glu Leu Lys Met Ser
755 760 765

Glu Ile Asn Ile Ser Asn Gly Ala Asn Phe Thr Leu Asn Ser His Val
770 775 780

Arg Gly Asp Asp Ala Phe Lys Ile Asn Lys Asp Leu Thr Ile Asn Ala
785 790 795 800

Thr Asn Ser Asn Phe Ser Leu Arg Gln Thr Lys Asp Asp Phe Tyr Asp
805 810 815

Gly Tyr Ala Arg Asn Ala Ile Asn Ser Thr Tyr Asn Ile Ser Ile Leu
820 825 830

Gly Gly Asn Val Thr Leu Gly Gly Gln Asn Ser Ser Ser Ser Ile Thr
835 840 845

Gly Asn Ile Thr Ile Glu Lys Ala Ala Asn Val Thr Leu Glu Ala Asn
850 855 860

Asn Ala Pro Asn Gln Gln Asn Ile Arg Asp Arg Val Ile Lys Leu Gly
865 870 875 880

Ser Leu Leu Val Asn Gly Ser Leu Ser Leu Thr Gly Glu Asn Ala Asp
885 890 895

Ile Lys Gly Asn Leu Thr Ile Ser Glu Ser Ala Thr Phe Lys Gly Lys
900 905 910

Thr Arg Asp Thr Leu Asn Ile Thr Gly Asn Phe Thr Asn Asn Gly Thr
915 920 925

Ala Glu Ile Asn Ile Thr Gln Gly Val Val Lys Leu Gly Asn Val Thr
930 935 940

Asn Asp Gly Asp Leu Asn Ile Thr Thr His Ala Lys Arg Asn Gln Arg
945 950 955 960

Ser Ile Ile Gly Gly Asp Ile Ile Asn Lys Lys Gly Ser Leu Asn Ile
965 970 975

Thr Asp Ser Asn Asn Asp Ala Glu Ile Gln Ile Gly Gly Asn Ile Ser
980 985 990

Gln Lys Glu Gly Asn Leu Thr Ile Ser Ser Asp Lys Ile Asn Ile Thr
995 1000 1005

Lys Gln Ile Thr Ile Lys Lys Gly Ile Asp Gly Glu Asp Ser Ser Ser
1010 1015 1020

Asp Ala Thr Ser Asn Ala Asn Leu Thr Ile Lys Thr Lys Glu Leu Lys
1025 1030 1035 1040

Leu Thr Glu Asp Leu Ser Ile Ser Gly Phe Asn Lys Ala Glu Ile Thr
1045 1050 1055

Ala Lys Asp Gly Arg Asp Leu Thr Ile Gly Asn Ser Asn Asp Gly Asn
1060 1065 1070

Ser Gly Ala Glu Ala Lys Thr Val Thr Phe Asn Asn Val Lys Asp Ser
1075 1080 1085

Lys Ile Ser Ala Asp Gly His Asn Val Thr Leu Asn Ser Lys Val Lys
1090 1095 1100

Thr Ser Ser Ser Asn Gly Gly Arg Glu Ser Asn Ser Asp Asn Asp Thr
1105 1110 1115 1120

Gly Leu Thr Ile Thr Ala Lys Asn Val Glu Val Asn Lys Asp Ile Thr
1125 1130 1135

Ser Leu Lys Thr Val Asn Ile Thr Ala Ser Glu Lys Val Thr Thr Thr
1140 1145 1150

Ala Gly Ser Thr Ile Asn Ala Thr Asn Gly Lys Ala Ser Ile Thr Thr
1155 1160 1165

Lys Thr Gly Asp Ile Ser Gly Thr Ile Ser Gly Asn Thr Val Ser Val
1170 1175 1180

Ser Ala Thr Val Asp Leu Thr Thr Lys Ser Gly Ser Lys Ile Glu Ala
1185 1190 1195 1200

Lys Ser Gly Glu Ala Asn Val Thr Ser Ala Thr Gly Thr Ile Gly Gly
1205 1210 1215

Thr Ile Ser Gly Asn Thr Val Asn Val Thr Ala Asn Ala Gly Asp Leu
1220 1225 1230

Thr Val Gly Asn Gly Ala Glu Ile Asn Ala Thr Glu Gly Ala Ala Thr
1235 1240 1245

Leu Thr Ala Thr Gly Asn Thr Leu Thr Thr Glu Ala Gly Ser Ser Ile
1250 1255 1260

Thr Ser Thr Lys Gly Gln Val Asp Leu Leu Ala Gln Asn Gly Ser Ile
1265 1270 1275 1280

Ala Gly Ser Ile Asn Ala Ala Asn Val Thr Leu Asn Thr Thr Gly Thr
1285 1290 1295

Leu Thr Thr Val Ala Gly Ser Asp Ile Lys Ala Thr Ser Gly Thr Leu
1300 1305 1310

Val Ile Asn Ala Lys Asp Ala Lys Leu Asn Gly Asp Ala Ser Gly Asp
1315 1320 1325

Ser Thr Glu Val Asn Ala Val Asn Ala Ser Gly Ser Gly Ser Val Thr
1330 1335 1340

Ala Ala Thr Ser Ser Ser Val Asn Ile Thr Gly Asp Leu Asn Thr Val
1345 1350 1355 1360

Asn Gly Leu Asn Ile Ile Ser Lys Asp Gly Arg Asn Thr Val Arg Leu
1365 1370 1375

Arg Gly Lys Glu Ile Glu Val Lys Tyr Ile Gln Pro Gly Val Ala Ser
1380 1385 1390

Val Glu Glu Val Ile Glu Ala Lys Arg Val Leu Glu Lys Val Lys Asp
1395 1400 1405

Leu Ser Asp Glu Glu Arg Glu Thr Leu Ala Lys Leu Gly Val Ser Ala
1410 1415 1420

Val Arg Phe Val Glu Pro Asn Asn Thr Ile Thr Val Asn Thr Gln Asn
1425 1430 1435 1440

Glu Phe Thr Thr Arg Pro Ser Ser Gln Val Ile Ile Ser Glu Gly Lys
1445 1450 1455

Ala Cys Phe Ser Ser Gly Asn Gly Ala Arg Val Cys Thr Asn Val Ala
1460 1465 1470

Asp Asp Gly Gln Pro
1475

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9171 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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ACAGCGTTCT CTTAATACTA GTACAAACCC ACAATAAAAT ATGACAAACA ACAATTACAA      60
CACCTTTTTT GCAGTCTATA TGCAAATATT TTAAAAAATA GTATAAATCC GCCATATAAA     120
ATGGTATAAT CTTTCATCTT TCATCTTTCA TCTTTCATCT TTCATCTTTC ATCTTTCATC     180
TTTCATCTTT CATCTTTCAT CTTTCATCTT TCATCTTTCA TCTTTCATCT TTCATCTTTC     240
ACATGAAATG ATGAACCGAG GGAAGGGAGG GAGGGGCAAG AATGAAGAGG GAGCTGAACG     300
AACGCAAATG ATAAAGTAAT TTAATTGTTC AACTAACCTT AGGAGAAAAT ATGAACAAGA     360
TATATCGTCT CAAATTCAGC AAACGCCTGA ATGCTTTGGT TGCTGTGTCT GAATTGGCAC     420
GGGGTTGTGA CCATTCCACA GAAAAAGGCA GCGAAAAACC TGCTCGCATG AAAGTGCGTC     480
ACTTAGCGTT AAAGCCACTT TCCGCTATGT TACTATCTTT AGGTGTAACA TCTATTCCAC     540
AATCTGTTTT AGCAAGCGGC TTACAAGGAA TGGATGTAGT ACACGGCACA GCCACTATGC     600
AAGTAGATGG TAATAAAACC ATTATCCGCA ACAGTGTTGA CGCTATCATT AATTGGAAAC     660
AATTTAACAT CGACCAAAAT GAAATGGTGC AGTTTTTACA AGAAAACAAC AACTCCGCCG     720
TATTCAACCG TGTTACATCT AACCAAATCT CCCAATTAAA AGGGATTTTA GATTCTAACG     780
GACAAGTCTT TTTAATCAAC CCAAATGGTA TCACAATAGG TAAAGACGCA ATTATTAACA     840
CTAATGGCTT TACGGCTTCT ACGCTAGACA TTTCTAACGA AAACATCAAG GCGCGTAATT     900
TCACCTTCGA GCAAACCAAA GATAAAGCGC TCGCTGAAAT TGTGAATCAC GGTTTAATTA     960
CTGTCGGTAA AGACGGCAGT GTAAATCTTA TTGGTGGCAA AGTGAAAAAC GAGGGTGTGA    1020
TTAGCGTAAA TGGTGGCAGC ATTTCTTTAC TCGCAGGGCA AAAAATCACC ATCAGCGATA    1080
TAATAAACCC AACCATTACT TACAGCATTG CCGCGCCTGA AAATGAAGCG GTCAATCTGG    1140
GCGATATTTT TGCCAAAGGC GGTAACATTA ATGTCCGTGC TGCCACTATT CGAAACCAAG    1200
CTTTCCGCCA AAGAGGGTGA AGCGGAAATT GGCGGTGTAA TTTCCGCTCA AAATCAGCAA    1260
GCTAAAGGCG GCAAGCTGAT GATTACAGGC GATAAAGTCA CATTAAAAAC AGGTGCAGTT    1320
ATCGACCTTT CAGGTAAAGA AGGGGGAGAA ACTTACCTTG GCGGTGACGA GCGCGGCGAA    1380
GGTAAAAACG GCATTCAATT AGCAAAGAAA ACCTCTTTAG AAAAAGGCTC AACCATCAAT    1440
GTATCAGGCA AAGAAAAAGG CGGACGCGCT ATTGTGTGGG GCGATATTGC GTTAATTGAC    1500
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GGCAATATTA ACGCTCAAGG TAGTGGTGAT ATCGCTAAAA CCGGTGGTTT TGTGGAGACG 1560
TCGGGGCATG ATTTATTCAT CAAAGACAAT GCAATTGTTG ACGCCAAAGA GTGGTTGTTA 1620
GACCCGGATA ATGTATCTAT TAATGCAGAA ACAGCAGGAC GCAGCAATAC TTCAGAAGAC 1680
GATGAATACA CGGGATCCGG GAATAGTGCC AGCACCCCAA AACGAAACAA AGAAAAGACA 1740
ACATTAACAA ACACAACTCT TGAGAGTATA CTAAAAAAAG GTACCTTTGT TAACATCACT 1800
GCTAATCAAC GCATCTATGT CAATAGCTCC ATTAATTTAT CCAATGGCAG CTTAACTCTT 1860
TGGAGTGAGG GTCGGAGCGG TGGCGGCGTT GAGATTAACA ACGATATTAC CACCGGTGAT 1920
GATACCAGAG GTGCAAACTT AACAATTTAC TCAGGCGGCT GGGTTGATGT TCATAAAAAT 1980
ATCTCACTCG GGGCGCAAGG TAACATAAAC ATTACAGCTA AACAAGATAT CGCCTTTGAG 2040
AAAGGAAGCA ACCAAGTCAT TACAGGTCAA GGGACTATTA CCTCAGGCAA TCAAAAAGGT 2100
TTTAGATTTA ATAATGTCTC TCTAAACGGC ACTGGCAGCG GACTGCAATT CACCACTAAA 2160
AGAACCAATA AATACGCTAT CACAAATAAA TTTGAAGGGA CTTTAAATAT TTCAGGGAAA 2220
GTGAACATCT CAATGGTTTT ACCTAAAAAT GAAAGTGGAT ATGATAAATT CAAAGGACGC 2280
ACTTACTGGA ATTTAACCTC GAAAGTGGAT ATGATAAATT CAAAGGACGC CCTCACTATT 2340
GACTCCAGAG GAAGCGATAG TGCAGGCACA CTTACCCAGC CTTATAATTT AAACGGTATA 2400
TCATTCAACA AAGACACTAC CTTTAATGTT GAACGAAATG CAAGAGTCAA CTTTGACATC 2460
AAGGCACCAA TAGGGATAAA TAAGTATTCT AGTTTGAATT ACGCATCATT TAATGGAAAC 2520
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CAAACCCCCG GTGTAGTTAT AAATTCTAAA TACTTTAATG TTTCAACAGG GTCAAGTTTA 2640
AGATTTAAAA CTTCAGGCTC AACAAAAACT GGCTTCTCAA TAGAGAAAGA TTTAACTTTA 2700
AATGCCACCG GAGGCAACAT AACACTTTTG CAAGTTGAAG GCACCGATGG AATGATTGGT 2760
AAAGGCATTG TAGCCAAAAA AAACATAACC TTTGAAGGAG GTAAGATGAG GTTTGGCTCC 2820
AGGAAAGCCG TAACAGAAAT CGAAGGCAAT GTTACTATCA ATAACAACGC TAACGTCACT 2880
CTTATCGGTT CGGATTTTGA CAACCATCAA AAACCTTTAA CTATTAAAAA AGATGTCATC 2940
ATTAATAGCG GCAACCTTAC CGCTGGAGGC AATATTGTCA ATATAGCCGG AAATCTTACC 3000
GTTGAAAGTA ACGCTAATTT CAAAGCTATC ACAAATTTCA CTTTTAATGT AGGCGGCTTG 3060
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ATTGATAATT CCAAGAATTT AAGCATCACC ACCAACTCCA GCTCCACTTA CCGCACTATT 3180
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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9323 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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CAATCCACCA ACAACTTTAC CGTTGGTTTT AAGCGTTAAT GTAAGTTCTT GCTCTTCTTG 240
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GTGAAGATTG TTTCAGCGAA ACCCTTTTAC GCTTACCCAA AGATGCCCTA CCTTATGTAC 8460
CTTCTGCACT CGCCCCACAA AAAGTGGATT ATGTACTCAG GGAAAACCCT GAAGTAGTCA 8520
ATATCGGTAT TGCCGCTACC ACAATGAAAT TAAACCCTGA ATTTTTGCTA ACATTGCAAG 8580
AAATCAGAGA TAAAGCTAAA GTCAAAATAC ATTTTCATTT CGCACTTGGA CAATCAACAG 8640
GCTTGACACA CCCTTATGTC AAATGGTTTA TCGAAAGCTA TTTAGGTGAC GATGCCACTG 8700
CACATCCCCA CGCACCTTAT CACGATTATC TGGCAATATT GCGTGATTGC GATATGCTAC 8760
TAAATCCGTT TCCTTTCGGT AATACTAACG GCATAATTGA TATGGTTACA TTAGGTTTAG 8820
TTGGTGTATG CAAAACGGGG GATGAAGTAC ATGAACATAT TGATGAAGGT CTGTTTAAAC 8880
GCTTAGGACT ACCAGAATGG CTGATAGCCG ACACACGAGA AACATATATT GAATGTGCTT 8940
TGCGTCTAGC AGAAAACCAT CAAGAACGCC TTGAACTCCG TCGTTACATC ATAGAAAACA 9000
ACGGCTTACA AAAGCTTTTT ACAGGCGACC CTCGTCCATT GGGCAAAATA CTGCTTAAGA 9060
AAACAAATGA ATGGAAGCGG AAGCACTTGA GTAAAAAATA ACGGTTTTTT AAAGTAAAAG 9120
TGCGGTTAAT TTTCAAAGCG TTTTAAAAAC CTCTCAAAAA TCAACCGCAC TTTTATCTTT 9180
ATAACGATCC CGCACGCTGA CAGTTTATCA GCCTCCCGCC ATAAAACTCC GCCTTTCATG 9240
GCGGAGATTT TAGCCAAAAC TGGCAGAAAT TAAAGGCTAA AATCACCAAA TTGCACCACA 9300
AAATCACCAA TACCCACAAA AAA 9323

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4794 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGAACAAGA TATATCGTCT CAAATTCAGC AAACGCCTGA ATGCTTTGGT TGCTGTGTCT 60
GAATTGACAC GGGGTTGTGA CCATTCCACA GAAAAAGGCA GTGAAAAACC TGTTCGTACG 120
AAAGTACGCC ACTTGGCGTT AAAGCCACTT TCCGCTATAT TGCTATCTTT GGGCATGGCA 180
TCCATTCCGC AATCTGTTTT AGCGAGCGGT TTACAGGGAA TGAGCGTCGT ACACGGTACA 240
GCAACCATGC AAGTAGACGG CAATAAAACC ACTATCCGTA ATAGCGTCAA TGCTATCATC 300
AATTGGAAAC AATTTAACAT TGACCAAAAT GAAATGGTGC AGTTTTTACA AGAAAGCAGC 360
AACTCTGCCG TTTTCAACCG TGTTACATCT GACCAAATCT CCCAATTAAA AGGGATTTTA 420

GATTCTAACG GACAAGTCTT TTTAATCAAC CCAAATGGTA TCACAATAGG TAAAGACGCA 480
ATTATTAACA CTAATGGCTT TACTGCTTCT ACGCTAGACA TTTCTAACGA AAACATCAAG 540
GCGCGTAATT TCACCCTTGA GCAAACCAAG GATAAAGCAC TCGCTGAAAT CGTGAATCAC 600
GGTTTAATTA CCGTTGGTAA AGACGGTAGC GTAAACCTTA TTGGTGGCAA AGTGAAAAAC 660
GAGGGCGTGA TTAGCGTAAA TGGCGGTAGT ATTTCTTTAC TTGCAGGGCA AAAAATCACC 720
ATCAGCGATA TAATAAATCC AACCATCACT TACAGCATTG CTGCACCTGA AAACGAAGCG 780
ATCAATCTGG GCGATATTTT TGCCAAAGGT GGTAACATTA ATGTCCGCGC TGCCACTATT 840
CGCAATAAAG GTAAACTTTC TGCCGACTCT GTAAGCAAAG ATAAAAGTGG TAACATTGTT 900
CTCTCTGCCA AAGAAGGTGA AGCGGAAATT GGCGGTGTAA TTTCCGCTCA AAATCAGCAA 960
GCCAAAGGTG GTAAGTTGAT GATTACAGGC GATAAAGTTA CATTGAAAAC GGGTGCAGTT 1020
ATCGACCTTT CGGGTAAAGA AGGGGGAGAA ACTTATCTTG GCGGTGACGA GCGTGGCGAA 1080
GGTAAAAACG GCATTCAATT AGCAAAGAAA ACCACTTTAG AAAAAGGCTC AACAATTAAT 1140
GTGTCAGGTA AAGAAAAAGG TGGGCGCGCT ATTGTATGGG GCGATATTGC GTTAATTGAC 1200
GGCAATATTA ATGCCCAAGG TAAAGATATC GCTAAAACTG GTGGTTTTGT GGAGACGTCG 1260
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CCAGAGAATG TGACTATTGA AGCTCCTTCC GCTTCTCGCG TCGAGCTGGG TGCCGATAGG 1380
AATTCCCACT CGGCAGAGGT GATAAAAGTG ACCCTAAAAA AAAATAACAC CTCCTTGACA 1440
ACACTAACCA ATACAACCAT TTCAAATCTT CTGAAAAGTG CCCACGTGGT GAACATAACG 1500
GCAAGGAGAA AACTTACCGT TAATAGCTCT ATCAGTATAG AAAGAGGCTC CCACTTAATT 1560
CTCCACAGTG AAGGTCAGGG CGGTCAAGGT GTTCAGATTG ATAAAGATAT TACTTCTGAA 1620
GGCGGAAATT TAACCATTTA TTCTGGCGGA TGGGTTGATG TTCATAAAAA TATTACGCTT 1680
GGTAGCGGCT TTTTAAACAT CACAACTAAA GAAGGAGATA TCGCCTTCGA AGACAAGTCT 1740
GGACGGAACA ACCTAACCAT TACAGCCCAA GGGACCATCA CCTCAGGTAA TAGTAACGGC 1800
TTTAGATTTA ACAACGTCTC TCTAAACAGC CTTGGCGGAA AGCTGAGCTT TACTGACAGC 1860
AGAGAGGACA GAGGTAGAAG AACTAAGGGT AATATCTCAA ACAAATTTGA CGGAACGTTA 1920
AACATTTCCG GAACTGTAGA TATCTCAATG AAAGCACCCA AAGTCAGCTG GTTTTACAGA 1980
GACAAAGGAC GCACCTACTG GAACGTAACC ACTTTAAATG TTACCTCGGG TAGTAAATTT 2040
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TTAAATGGCA TAACATTTAA TAAAGCCACT TTTAATATCG CACAAGGCTC AACAGCTAAC 2160
TTTAGCATCA AGGCATCAAT AATGCCCTTT AAGAGTAACG CTAACTACGC ATTATTTAAT 2220
GAAGATATTT CAGTCTCAGG GGGGGGTAGC CTTAATTTCA AACTTAACGC CTCATCTAGC 2280
AACATACAAA CCCCTGGCGT AATTATAAAA TCTCAAAACT TTAATGTCTC AGGAGGGTCA 2340
ACTTTAAATC TCAAGGCTGA AGGTTCAACA GAAACCGCTT TTTCAATAGA AAATGATTTA 2400
AACTTAAACG CCACCGGTGG CAATATAACA ATCAGACAAG TCGAGGGTAC CGATTCACGC 2460

GTCAACAAAG GTGTCGCAGC CAAAAAAAAC ATAACTTTTA AAGGGGGTAA TATCACCTTC 2520
GGCTCTCAAA AAGCCACAAC AGAAATCAAA GGCAATGTTA CCATCAATAA AAACACTAAC 2580
GCTACTCTTT GTGGTGCGAA TTTTGCCGAA AACAAATCGC CTTTAAATAT AGCAGGAAAT 2640
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TCATTTGACA ACAATGGCGC TTCAAACATT TCCATTGCCA GAGGAGGGGC TAAATTTAAA 2820
GATATCAATA ACACCAGTAG CTTAAATATT ACCACCAACT CTGATACCAC TTACCGCACC 2880
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GACGCTGAAA TCCAAATTGG CGGCAATATC TCACAAAAAG AAGGCAATCT CACAATTTCT 3000
TCTGATAAAG TAAATATTAC CAATCAGATA ACAATCAAAG CAGGCGTTGA AGGGGGGCGT 3060
TCTGATTCAA GTGAGGCAGA AAATGCTAAC CTAACTATTC AAACCAAAGA GTTAAAATTG 3120
GCAGGAGACC TAAATATTTC AGGCTTTAAT AAAGCAGAAA TTACAGCTAA AAATGGCAGT 3180
GATTTAACTA TTGGCAATGC TAGCGGTGGT AATGCTGATG CTAAAAAAGT GACTTTTGAC 3240
AAGGTTAAAG ATTCAAAAAT CTCGACTGAC GGTCACAATG TAACACTAAA TAGCGAAGTG 3300
AAAACGTCTA ATGGTAGTAG CAATGCTGGT AATGATAACA GCACCGGTTT AACCATTTCC 3360
GCAAAAGATG TAACGGTAAA CAATAACGTT ACCTCCCACA AGACAATAAA TATCTCTGCC 3420
GCAGCAGGAA ATGTAACAAC CAAAGAAGGC ACAACTATCA ATGCAACCAC AGGCAGCGTG 3480
GAAGTAACTG CTCAAAATGG TACAATTAAA GGCAACATTA CCTCGCAAAA TGTAACAGTG 3540
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GTAAACATTA GTACAAAAAC AGGGGATATT AAAGGTGGAA TTGAATCAAC TTCCGGTAAT 3660
GTAAATATTA CAGCGAGCGG CAATACACTT AAGGTAAGTA ATATCACTGG TCAAGATGTA 3720
ACAGTAACAG CGGATGCAGG AGCCTTGACA ACTACAGCAG GCTCAACCAT TAGTGCGACA 3780
ACAGGCAATG CAAATATTAC AACCAAAACA GGTGATATCA ACGGTAAAGT TGAATCCAGC 3840
TCCGGCTCTG TAACACTTGT TGCAACTGGA GCAACTCTTG CTGTAGGTAA TATTTCAGGT 3900
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GGTAATACAG TAAATGTTAC AGCAAGCACT GGTGATTTAA CTATTGGAAA TAGTGCAAAA 4080
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ACAGGCTCTA GCATTACCTC AAGCAATGGT CAGACAACTC TTACAGCCAA GGATAGCAGT 4200
ATCGCAGGAA ACATTAATGC TGCTAATGTG ACGTTAAATA CCACAGGCAC TTTAACTACT 4260
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AAATTAGATG GTGCTGCATC AGGTGACCGC ACAGTAGTAA ATGCAACTAA CGCAAGTGGC 4380
TCTGGTAACG TGACTGCGAA AACCTCAAGC AGCGTGAATA TCACCGGGGA TTTAAACACA 4440
ATAAATGGGT TAAATATCAT TTCGGAAAAT GGTAGAAACA CTGTGCGCTT AAGAGGCAAG 4500

GAAATTGATG TGAAATATAT CCAACCAGGT GTAGCAAGCG TAGAAGAGGT AATTGAAGCG 4560

AAACGCGTCC TTGAGAAGGT AAAAGATTTA TCTGATGAAG AAAGAGAAAC ACTAGCCAAA 4620

CTTGGTGTAA GTGCTGTACG TTTCGTTGAG CCAAATAATG CCATTACGGT TAATACACAA 4680

AACGAGTTTA CAACCAAACC ATCAAGTCAA GTGACAATTT CTGAAGGTAA GGCGTGTTTC 4740

TCAAGTGGTA ATGGCGCACG AGTATGTACC AATGTTGCTG ACGATGGACA GCAG 4794

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4803 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGAACAAGA TATATCGTCT CAAATTCAGC AAACGCCTGA ATGCTTTGGT TGCTGTGTCT 60

GAATTGACAC GGGGTTGTGA CCATTCCACA GAAAAAGGCA GTGAAAAACC TGTTCGTACG 120

AAAGTACGCC ACTTGGCGTT AAAGCCACTT TCCGCTATAT TGCTATCTTT GGGCATGGCA 180

TCCATTCCGC AATCTGTTTT AGCGAGCGGT TTACAGGGAA TGAGCGTCGT ACACGGTACA 240

GCAACCATGC AAGTAGACGG CAATAAAACC ACTATCCGTA ATAGCGTCAA TGCTATCATC 300

AATTGGAAAC AATTTAACAT TGACCAAAAT GAAATGGTGC AGTTTTTACA AGAAAGCAGC 360

AACTCTGCCG TTTTCAACCG TGTTACATCT GACCAAATCT CCCAATTAAA AGGGATTTTA 420

GATTCTAACG GACAAGTCTT TTTAATCAAC CCAAATGGTA TCACAATAGG TAAAGACGCA 480

ATTATTAACA CTAATGGCTT TACTGCTTCT ACGCTAGACA TTTCTAACGA AAACATCAAG 540

GCGCGTAATT TCACCCTTGA GCAAACCAAG GATAAAGCAC TCGCTGAAAT CGTGAATCAC 600

GGTTTAATTA CCGTTGGTAA AGACGGTAGC GTAAACCTTA TTGGTGGCAA AGTGAAAAAC 660

GAGGGCGTGA TTAGCGTAAA TGGCGGTAGT ATTTCTTTAC TTGCAGGGCA AAAAATCACC 720

ATCAGCGATA TAATAAATCC AACCATCACT TACAGCATTG CTGCACCTGA AAACGAAGCG 780

ATCAATCTGG GCGATATTTT TGCCAAAGGT GGTAACATTA ATGTCCGCGC TGCCACTATT 840

CGCAATAAAG GTAAACTTTC TGCCGACTCT GTAAGCAAAG ATAAAAGTGG TAACATTGTT 900

CTCTCTGCCA AAGAAGGTGA AGCGGAAATT GGCGGTGTAA TTTCCGCTCA AAATCAGCAA 960

GCCAAAGGTG GTAAGTTGAT GATTACAGGT GATAAAGTCA CATTAAAAAC AGGTGCAGTT 1020

ATCGACCTTT CAGGTAAAGA AGGGGGAGAG ACTTATCTTG GCGGTGATGA GCGTGGCGAA 1080

GGTAAAAATG GTATTCAATT AGCGAAGAAA ACCTCTTTAG AAAAAGGCTC GACAATTAAT 1140

GTATCAGGCA AAGAAAAAGG CGGGCGCGCT ATTGTATGGG GCGATATTGC ATTAATTAAT 1200

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CCAGATGATG TGTCCATTGA AACTCTTACA TCTGGACGCA ATAATACCGG CGAAAACCAA 1380
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CACACTAAAC GAGATGGAGT TAAAATTAAC GGTGATATTA CCTCAAACGA AAATGGTAAT 1620
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ACCATTTCCG CAAAAGATGT AACGGTAAAC AATAACGTTA CCTCCCACAA GACAATAAAT 3420

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GGCAGCGTGG AAGTAACTGC TCAAAATGGT ACAATTAAAG GCAACATTAC CTCGCAAAAT 3540

GTAACAGTGA CAGCAACAGA AAATCTTGTT ACCACAGAGA ATGCTGTCAT TAATGCAACC 3600

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GCGTGTTTCT CAAGTGGTAA TGGCGCACGA GTATGTACCA ATGTTGCTGA CGATGGACAG 4800

CAG 4803

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1599 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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Met Asn Lys Ile Tyr Arg Leu Lys Phe Ser Lys Arg Leu Asn Ala Leu
1               5                   10                  15

Val Ala Val Ser Glu Leu Thr Arg Gly Cys Asp His Ser Thr Glu Lys
            20                  25                  30

Gly Ser Glu Lys Pro Val Arg Thr Lys Val Arg His Leu Ala Leu Lys
        35                  40                  45

Pro Leu Ser Ala Ile Leu Leu Ser Leu Gly Met Ala Ser Ile Pro Gln
    50                  55                  60

Ser Val Leu Ala Ser Gly Leu Gln Gly Met Ser Val Val His Gly Thr
65                  70                  75                  80

Ala Thr Met Gln Val Asp Gly Asn Lys Thr Thr Ile Arg Asn Ser Val
                85                  90                  95

Asn Ala Ile Ile Asn Trp Lys Gln Phe Asn Ile Asp Gln Asn Glu Met
            100                 105                 110

Glu Gln Phe Leu Gln Glu Ser Ser Asn Ser Ala Val Phe Asn Arg Val
        115                 120                 125

Thr Ser Asp Gln Ile Ser Gln Leu Lys Gly Ile Leu Asp Ser Asn Gly
    130                 135                 140

Gln Val Phe Leu Ile Asn Pro Asn Gly Ile Thr Ile Gly Lys Asp Ala
145                 150                 155                 160

Ile Ile Asn Thr Asn Gly Phe Thr Ala Ser Thr Leu Asp Ile Ser Asn
                165                 170                 175

Glu Asn Ile Lys Ala Arg Asn Phe Thr Leu Glu Gln Thr Lys Asp Lys
            180                 185                 190

Ala Leu Ala Glu Ile Val Asn His Gly Leu Ile Thr Val Gly Lys Asp
        195                 200                 205

Gly Ser Val Asn Leu Ile Gly Gly Lys Val Lys Asn Glu Gly Val Ile
    210                 215                 220

Ser Val Asn Gly Gly Ser Ile Ser Leu Leu Ala Gly Gln Lys Ile Thr
225                 230                 235                 240

Ile Ser Asp Ile Ile Asn Pro Thr Ile Thr Tyr Ser Ile Ala Ala Pro
                245                 250                 255

Glu Asn Glu Ala Ile Asn Leu Gly Asp Ile Phe Ala Lys Gly Gly Asn
            260                 265                 270

Ile Asn Val Arg Ala Ala Thr Ile Arg Asn Lys Gly Lys Leu Ser Ala
        275                 280                 285

Asp Ser Val Ser Lys Asp Lys Ser Gly Asn Ile Val Leu Ser Ala Lys
    290                 295                 300

Glu Gly Glu Ala Glu Ile Gly Gly Val Ile Ser Ala Gln Asn Gln Gln
305                 310                 315                 320

Ala Lys Gly Gly Lys Leu Met Ile Thr Gly Asp Lys Val Thr Leu Lys
                325                 330                 335
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Thr Gly Ala Val Ile Asp Leu Ser Gly Lys Glu Gly Gly Glu Thr Tyr
340 345 350

Leu Gly Gly Asp Glu Arg Gly Glu Gly Lys Asn Gly Ile Gln Leu Ala
355 360 365

Lys Lys Thr Thr Leu Glu Lys Gly Ser Thr Ile Asn Val Ser Gly Lys
370 375 380

Glu Lys Gly Gly Arg Ala Ile Val Trp Gly Asp Ile Ala Leu Ile Asp
385 390 395 400

Gly Asn Ile Asn Ala Gln Gly Lys Asp Ile Ala Lys Thr Gly Gly Phe
405 410 415

Val Glu Thr Ser Gly His Tyr Leu Ser Ile Asp Asp Asn Ala Ile Val
420 425 430

Lys Thr Lys Glu Trp Leu Leu Asp Pro Glu Asn Val Thr Ile Glu Ala
435 440 445

Pro Ser Ala Ser Arg Val Glu Leu Gly Ala Asp Arg Asn Ser His Ser
450 455 460

Ala Glu Val Ile Lys Val Thr Leu Lys Lys Asn Asn Thr Ser Leu Thr
465 470 475 480

Thr Leu Thr Asn Thr Thr Ile Ser Asn Leu Leu Lys Ser Ala His Val
485 490 495

Val Asn Ile Thr Ala Arg Arg Lys Leu Thr Val Asn Ser Ser Ile Ser
500 505 510

Ile Glu Arg Gly Ser His Leu Ile Leu His Ser Glu Gly Gln Gly Gly
515 520 525

Gln Gly Val Gln Ile Asp Lys Asp Ile Thr Ser Glu Gly Gly Asn Leu
530 535 540

Thr Ile Tyr Ser Gly Gly Trp Val Asp Val His Lys Asn Ile Thr Leu
545 550 555 560

Gly Ser Gly Phe Leu Asn Ile Thr Thr Lys Glu Gly Asp Ile Ala Phe
565 570 575

Glu Asp Lys Ser Gly Arg Asn Asn Leu Thr Ile Thr Ala Gln Gly Thr
580 585 590

Ile Thr Ser Gly Asn Ser Asn Gly Phe Arg Phe Asn Asn Val Ser Leu
595 600 605

Asn Ser Leu Gly Gly Lys Leu Ser Phe Thr Asp Ser Arg Glu Asp Arg
610 615 620

Gly Arg Arg Thr Lys Gly Asn Ile Ser Asn Lys Phe Asp Gly Thr Leu
625 630 635 640

Asn Ile Ser Gly Thr Val Asp Ile Ser Met Lys Ala Pro Lys Val Ser
645 650 655

Trp Phe Tyr Arg Asp Lys Gly Arg Thr Tyr Trp Asn Val Thr Thr Leu
660 665 670

Asn Val Thr Ser Gly Ser Lys Phe Asn Leu Ser Ile Asp Ser Thr Gly
675 680 685

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Ser Gly Ser Thr Gly Pro Ser Ile Arg Asn Ala Glu Leu Asn Gly Ile
    690                 695                 700

Thr Phe Asn Lys Ala Thr Phe Asn Ile Ala Gln Gly Ser Thr Ala Asn
705                 710                 715                 720

Phe Ser Ile Lys Ala Ser Ile Met Pro Phe Lys Ser Asn Ala Asn Tyr
                725                 730                 735

Ala Leu Phe Asn Glu Asp Ile Ser Val Ser Gly Gly Gly Ser Val Asn
            740                 745                 750

Phe Lys Leu Asn Ala Ser Ser Ser Asn Ile Gln Thr Pro Gly Val Ile
        755                 760                 765

Ile Lys Ser Gln Asn Phe Asn Val Ser Gly Gly Ser Thr Leu Asn Leu
    770                 775                 780

Lys Ala Glu Gly Ser Thr Glu Thr Ala Phe Ser Ile Glu Asn Asp Leu
785                 790                 795                 800

Asn Leu Asn Ala Thr Gly Gly Asn Ile Thr Ile Arg Gln Val Glu Gly
                805                 810                 815

Thr Asp Ser Arg Val Asn Lys Gly Val Ala Ala Lys Lys Asn Ile Thr
            820                 825                 830

Phe Lys Gly Gly Asn Ile Thr Phe Gly Ser Gln Lys Ala Thr Thr Glu
        835                 840                 845

Ile Lys Gly Asn Val Thr Ile Asn Lys Asn Thr Asn Ala Thr Leu Arg
    850                 855                 860

Gly Ala Asn Phe Ala Glu Asn Lys Ser Pro Leu Asn Ile Ala Gly Asn
865                 870                 875                 880

Val Ile Asn Asn Gly Asn Leu Thr Thr Ala Gly Ser Ile Ile Asn Ile
                885                 890                 895

Ala Gly Asn Leu Thr Val Ser Lys Gly Ala Asn Leu Gln Ala Ile Thr
            900                 905                 910

Asn Tyr Thr Phe Asn Val Ala Gly Ser Phe Asp Asn Asn Gly Ala Ser
        915                 920                 925

Asn Ile Ser Ile Ala Arg Gly Gly Ala Lys Phe Lys Asp Ile Asn Asn
    930                 935                 940

Thr Ser Ser Leu Asn Ile Thr Thr Asn Ser Asp Thr Thr Tyr Arg Thr
945                 950                 955                 960

Ile Ile Lys Gly Asn Ile Ser Asn Lys Ser Gly Asp Leu Asn Ile Ile
                965                 970                 975

Asp Lys Lys Ser Asp Ala Glu Ile Gln Ile Gly Gly Asn Ile Ser Gln
            980                 985                 990

Lys Glu Gly Asn Leu Thr Ile Ser Ser Asp Lys Val Asn Ile Thr Asn
        995                 1000                1005

Gln Ile Thr Ile Lys Ala Gly Val Glu Gly Gly Arg Ser Asp Ser Ser
    1010                1015                1020

Glu Ala Glu Asn Ala Asn Leu Thr Ile Gln Thr Lys Glu Leu Lys Leu
1025                1030                1035                1040
```

Ala Gly Asp Leu Asn Ile Ser Gly Phe Asn Lys Ala Glu Ile Thr Ala
1045 1050 1055

Lys Asn Gly Ser Asp Leu Thr Ile Gly Asn Ala Ser Gly Gly Asn Ala
1060 1065 1070

Asp Ala Lys Lys Val Thr Phe Asp Lys Val Lys Asp Ser Lys Ile Ser
1075 1080 1085

Thr Asp Gly His Asn Val Thr Leu Asn Ser Glu Val Lys Thr Ser Asn
1090 1095 1100

Gly Ser Ser Asn Ala Gly Asn Asp Asn Ser Thr Gly Leu Thr Ile Ser
1105 1110 1115 1120

Ala Lys Asp Val Thr Val Asn Asn Asn Val Thr Ser His Lys Thr Ile
1125 1130 1135

Asn Ile Ser Ala Ala Ala Gly Asn Val Thr Thr Lys Glu Gly Thr Thr
1140 1145 1150

Ile Asn Ala Thr Thr Gly Ser Val Glu Val Thr Ala Gln Asn Gly Thr
1155 1160 1165

Ile Lys Gly Asn Ile Thr Ser Gln Asn Val Thr Val Thr Ala Thr Glu
1170 1175 1180

Asn Leu Val Thr Thr Glu Asn Ala Val Ile Asn Ala Thr Ser Gly Thr
1185 1190 1195 1200

Val Asn Ile Ser Thr Lys Thr Gly Asp Ile Lys Gly Gly Ile Glu Ser
1205 1210 1215

Thr Ser Gly Asn Val Asn Ile Thr Ala Ser Gly Asn Thr Leu Lys Val
1220 1225 1230

Ser Asn Ile Thr Gly Gln Asp Val Thr Val Thr Ala Asp Ala Gly Ala
1235 1240 1245

Leu Thr Thr Thr Ala Gly Ser Thr Ile Ser Ala Thr Thr Gly Asn Ala
1250 1255 1260

Asn Ile Thr Thr Lys Thr Gly Asp Ile Asn Gly Lys Val Glu Ser Ser
1265 1270 1275 1280

Ser Gly Ser Val Thr Leu Val Ala Thr Gly Ala Thr Leu Ala Val Gly
1285 1290 1295

Asn Ile Ser Gly Asn Thr Val Thr Ile Thr Ala Asp Ser Gly Lys Leu
1300 1305 1310

Thr Ser Thr Val Gly Ser Thr Ile Asn Gly Thr Asn Ser Val Thr Thr
1315 1320 1325

Ser Ser Gln Ser Gly Asp Ile Glu Gly Thr Ile Ser Gly Asn Thr Val
1330 1335 1340

Asn Val Thr Ala Ser Thr Gly Asp Leu Thr Ile Gly Asn Ser Ala Lys
1345 1350 1355 1360

Val Glu Ala Lys Asn Gly Ala Ala Thr Leu Thr Ala Glu Ser Gly Lys
1365 1370 1375

Leu Thr Thr Gln Thr Gly Ser Ser Ile Thr Ser Ser Asn Gly Gln Thr
1380 1385 1390

Thr Leu Thr Ala Lys Asp Ser Ser Ile Ala Gly Asn Ile Asn Ala Ala
1395 1400 1405

Asn Val Thr Leu Asn Thr Thr Gly Thr Leu Thr Thr Thr Gly Asp Ser
1410 1415 1420

Lys Ile Asn Ala Thr Ser Gly Thr Leu Thr Ile Asn Ala Lys Asp Ala
1425 1430 1435 1440

Lys Leu Asp Gly Ala Ala Ser Gly Asp Arg Thr Val Val Asn Ala Thr
1445 1450 1455

Asn Ala Ser Gly Ser Gly Asn Val Thr Ala Lys Thr Ser Ser Ser Val
1460 1465 1470

Asn Ile Thr Gly Asp Leu Asn Thr Ile Asn Gly Leu Asn Ile Ile Ser
1475 1480 1485

Glu Asn Gly Arg Asn Thr Val Arg Leu Arg Gly Lys Glu Ile Asp Val
1490 1495 1500

Lys Tyr Ile Gln Pro Gly Val Ala Ser Val Glu Glu Val Ile Glu Ala
1505 1510 1515 1520

Lys Arg Val Leu Glu Lys Val Lys Asp Leu Ser Asp Glu Glu Arg Glu
1525 1530 1535

Thr Leu Ala Lys Leu Gly Val Ser Ala Val Arg Phe Val Glu Pro Asn
1540 1545 1550

Asn Ala Ile Thr Val Asn Thr Gln Asn Glu Phe Thr Thr Lys Pro Ser
1555 1560 1565

Ser Gln Val Thr Ile Ser Glu Gly Lys Ala Cys Phe Ser Ser Gly Asn
1570 1575 1580

Gly Ala Arg Val Cys Thr Asn Val Ala Asp Asp Gly Gln Gln Pro
1585 1590 1595

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1600 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Asn Lys Ile Tyr Arg Leu Lys Phe Ser Lys Arg Leu Asn Ala Leu
1 5 10 15

Val Ala Val Ser Glu Leu Thr Arg Gly Cys Asp His Ser Thr Glu Lys
20 25 30

Gly Ser Glu Lys Pro Val Arg Thr Lys Val Arg His Leu Ala Leu Lys
35 40 45

Pro Leu Ser Ala Ile Leu Leu Ser Leu Gly Met Ala Ser Ile Pro Gln
50 55 60

Ser Val Leu Ala Ser Gly Leu Gln Gly Met Ser Val Val His Gly Thr
65 70 75 80

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Ala Thr Met Gln Val Asp Gly Asn Lys Thr Thr Ile Arg Asn Ser Val
                85                  90                  95

Asn Ala Ile Ile Asn Trp Lys Gln Phe Asn Ile Asp Gln Asn Glu Met
            100                 105                 110

Glu Gln Phe Leu Gln Glu Ser Ser Asn Ser Ala Val Phe Asn Arg Val
        115                 120                 125

Thr Ser Asp Gln Ile Ser Gln Leu Lys Gly Ile Leu Asp Ser Asn Gly
    130                 135                 140

Gln Val Phe Leu Ile Asn Pro Asn Gly Ile Thr Ile Gly Lys Asp Ala
145                 150                 155                 160

Ile Ile Asn Thr Asn Gly Phe Thr Ala Ser Thr Leu Asp Ile Ser Asn
                165                 170                 175

Glu Asn Ile Lys Ala Arg Asn Phe Thr Leu Glu Gln Thr Lys Asp Lys
            180                 185                 190

Ala Leu Ala Glu Ile Val Asn His Gly Leu Ile Thr Val Gly Lys Asp
        195                 200                 205

Gly Ser Val Asn Leu Ile Gly Gly Lys Val Lys Asn Glu Gly Val Ile
    210                 215                 220

Ser Val Asn Gly Gly Ser Ile Ser Leu Leu Ala Gly Gln Lys Ile Thr
225                 230                 235                 240

Ile Ser Asp Ile Ile Asn Pro Thr Ile Thr Tyr Ser Ile Ala Ala Pro
                245                 250                 255

Glu Asn Glu Ala Ile Asn Leu Gly Asp Ile Phe Ala Lys Gly Gly Asn
            260                 265                 270

Ile Asn Val Arg Ala Ala Thr Ile Arg Asn Lys Gly Lys Leu Ser Ala
        275                 280                 285

Asp Ser Val Ser Lys Asp Lys Ser Gly Asn Ile Val Leu Ser Ala Lys
    290                 295                 300

Glu Gly Glu Ala Glu Ile Gly Gly Val Ile Ser Ala Gln Asn Gln Gln
305                 310                 315                 320

Ala Lys Gly Gly Lys Leu Met Ile Thr Gly Asp Lys Val Thr Leu Lys
                325                 330                 335

Thr Gly Ala Val Ile Asp Leu Ser Gly Lys Glu Gly Gly Glu Thr Tyr
            340                 345                 350

Leu Gly Gly Asp Glu Arg Gly Glu Gly Lys Asn Gly Ile Gln Leu Ala
        355                 360                 365

Lys Lys Thr Thr Leu Glu Lys Gly Ser Thr Ile Asn Val Ser Gly Lys
    370                 375                 380

Glu Lys Gly Gly Arg Ala Ile Val Trp Gly Asp Ile Ala Leu Ile Asp
385                 390                 395                 400

Gly Asn Ile Asn Ala Gln Gly Ser Asp Ile Ala Lys Thr Gly Gly Phe
                405                 410                 415

Val Glu Thr Ser Gly His Asp Leu Ser Ile Gly Asp Asp Val Ile Val
            420                 425                 430
```

Asp Ala Lys Glu Trp Leu Leu Asp Pro Asp Asp Val Ser Ile Glu Thr
435 440 445

Leu Thr Ser Gly Arg Asn Asn Thr Gly Glu Asn Gln Gly Tyr Thr Thr
450 455 460

Gly Asp Gly Thr Lys Glu Ser Pro Lys Gly Asn Ser Ile Ser Lys Pro
465 470 475 480

Thr Leu Thr Asn Ser Thr Leu Glu Gln Ile Leu Arg Arg Gly Ser Tyr
485 490 495

Val Asn Ile Thr Ala Asn Asn Arg Ile Tyr Val Asn Ser Ser Ile Asn
500 505 510

Leu Ser Asn Gly Ser Leu Thr Leu His Thr Lys Arg Asp Gly Val Lys
515 520 525

Ile Asn Gly Asp Ile Thr Ser Asn Glu Asn Gly Asn Leu Thr Ile Lys
530 535 540

Ala Gly Ser Trp Val Asp Val His Lys Asn Ile Thr Leu Gly Thr Gly
545 550 555 560

Phe Leu Asn Ile Val Ala Gly Asp Ser Val Ala Phe Glu Arg Glu Gly
565 570 575

Asp Lys Ala Arg Asn Ala Thr Asp Ala Gln Ile Thr Ala Gln Gly Thr
580 585 590

Ile Thr Val Asn Lys Asp Asp Lys Gln Phe Arg Phe Asn Asn Val Ser
595 600 605

Leu Asn Gly Thr Gly Lys Gly Leu Lys Phe Ile Ala Asn Gln Asn Asn
610 615 620

Phe Thr His Lys Phe Asp Gly Glu Ile Asn Ile Ser Gly Ile Val Thr
625 630 635 640

Ile Asn Gln Thr Thr Lys Lys Asp Val Lys Tyr Trp Asn Ala Ser Lys
645 650 655

Asp Ser Tyr Trp Asn Val Ser Ser Leu Thr Leu Asn Thr Val Gln Lys
660 665 670

Phe Thr Phe Ile Lys Phe Val Asp Ser Gly Ser Asn Gly Gln Asp Leu
675 680 685

Arg Ser Ser Arg Arg Ser Phe Ala Gly Val His Phe Asn Gly Ile Gly
690 695 700

Gly Lys Thr Asn Phe Asn Ile Gly Ala Asn Ala Lys Ala Leu Phe Lys
705 710 715 720

Leu Lys Pro Asn Ala Ala Thr Asp Pro Lys Lys Glu Leu Pro Ile Thr
725 730 735

Phe Asn Ala Asn Ile Thr Ala Thr Gly Asn Ser Asp Ser Ser Val Met
740 745 750

Phe Asp Ile His Ala Asn Leu Thr Ser Arg Ala Ala Gly Ile Asn Met
755 760 765

Asp Ser Ile Asn Ile Thr Gly Gly Leu Asp Phe Ser Ile Thr Ser His
770 775 780

Asn Arg Asn Ser Asn Ala Phe Glu Ile Lys Lys Asp Leu Thr Ile Asn
785 790 795 800

Ala Thr Gly Ser Asn Phe Ser Leu Lys Gln Thr Lys Asp Ser Phe Tyr
805 810 815

Asn Glu Tyr Ser Lys His Ala Ile Asn Ser Ser His Asn Leu Thr Ile
820 825 830

Leu Gly Gly Asn Val Thr Leu Gly Gly Glu Asn Ser Ser Ser Ser Ile
835 840 845

Thr Gly Asn Ile Asn Ile Thr Asn Lys Ala Asn Val Thr Leu Gln Ala
850 855 860

Asp Thr Ser Asn Ser Asn Thr Gly Leu Lys Lys Arg Thr Leu Thr Leu
865 870 875 880

Gly Asn Ile Ser Val Glu Gly Asn Leu Ser Leu Thr Gly Ala Asn Ala
885 890 895

Asn Ile Val Gly Asn Leu Ser Ile Ala Glu Asp Ser Thr Phe Lys Gly
900 905 910

Glu Ala Ser Asp Asn Leu Asn Ile Thr Gly Thr Phe Thr Asn Asn Gly
915 920 925

Thr Ala Asn Ile Asn Ile Lys Gly Val Val Lys Leu Gly Asp Ile Asn
930 935 940

Asn Lys Gly Gly Leu Asn Ile Thr Thr Asn Ala Ser Gly Thr Gln Lys
945 950 955 960

Thr Ile Ile Asn Gly Asn Ile Thr Asn Glu Lys Gly Asp Leu Asn Ile
965 970 975

Lys Asn Ile Lys Ala Asp Ala Glu Ile Gln Ile Gly Gly Asn Ile Ser
980 985 990

Gln Lys Glu Gly Asn Leu Thr Ile Ser Ser Asp Lys Val Asn Ile Thr
995 1000 1005

Asn Gln Ile Thr Ile Lys Ala Gly Val Glu Gly Gly Arg Ser Asp Ser
1010 1015 1020

Ser Glu Ala Glu Asn Ala Asn Leu Thr Ile Gln Thr Lys Glu Leu Lys
1025 1030 1035 1040

Leu Ala Gly Asp Leu Asn Ile Ser Gly Phe Asn Lys Ala Glu Ile Thr
1045 1050 1055

Ala Lys Asn Gly Ser Asp Leu Thr Ile Gly Asn Ala Ser Gly Gly Asn
1060 1065 1070

Ala Asp Ala Lys Lys Val Thr Phe Asp Lys Val Lys Asp Ser Lys Ile
1075 1080 1085

Ser Thr Asp Gly His Asn Val Thr Leu Asn Ser Glu Val Lys Thr Ser
1090 1095 1100

Asn Gly Ser Ser Asn Ala Gly Asn Asp Asn Ser Thr Gly Leu Thr Ile
1105 1110 1115 1120

Ser Ala Lys Asp Val Thr Val Asn Asn Asn Val Thr Ser His Lys Thr
1125 1130 1135

Ile Asn Ile Ser Ala Ala Ala Gly Asn Val Thr Thr Lys Glu Gly Thr
1140 1145 1150

Thr Ile Asn Ala Thr Thr Gly Ser Val Glu Val Thr Ala Gln Asn Gly
1155 1160 1165

Thr Ile Lys Gly Asn Ile Thr Ser Gln Asn Val Thr Val Thr Ala Thr
1170 1175 1180

Glu Asn Leu Val Thr Thr Glu Asn Ala Val Ile Asn Ala Thr Ser Gly
1185 1190 1195 1200

Thr Val Asn Ile Ser Thr Lys Thr Gly Asp Ile Lys Gly Gly Ile Glu
1205 1210 1215

Ser Thr Ser Gly Asn Val Asn Ile Thr Ala Ser Gly Asn Thr Leu Lys
1220 1225 1230

Val Ser Asn Ile Thr Gly Gln Asp Val Thr Val Thr Ala Asp Ala Gly
1235 1240 1245

Ala Leu Thr Thr Thr Ala Gly Ser Thr Ile Ser Ala Thr Thr Gly Asn
1250 1255 1260

Ala Asn Ile Thr Thr Lys Thr Gly Asp Ile Asn Gly Lys Val Glu Ser
1265 1270 1275 1280

Ser Ser Gly Ser Val Thr Leu Val Ala Thr Gly Ala Thr Leu Ala Val
1285 1290 1295

Gly Asn Ile Ser Gly Asn Thr Val Thr Ile Thr Ala Asp Ser Gly Lys
1300 1305 1310

Leu Thr Ser Thr Val Gly Ser Thr Ile Asn Gly Thr Asn Ser Val Thr
1315 1320 1325

Thr Ser Ser Gln Ser Gly Asp Ile Glu Gly Thr Ile Ser Gly Asn Thr
1330 1335 1340

Val Asn Val Thr Ala Ser Thr Gly Asp Leu Thr Ile Gly Asn Ser Ala
1345 1350 1355 1360

Lys Val Glu Ala Lys Asn Gly Ala Ala Thr Leu Thr Ala Glu Ser Gly
1365 1370 1375

Lys Leu Thr Thr Gln Thr Gly Ser Ser Ile Thr Ser Ser Asn Gly Gln
1380 1385 1390

Thr Thr Leu Thr Ala Lys Asp Ser Ser Ile Ala Gly Asn Ile Asn Ala
1395 1400 1405

Ala Asn Val Thr Leu Asn Thr Thr Gly Thr Leu Thr Thr Thr Gly Asp
1410 1415 1420

Ser Lys Ile Asn Ala Thr Ser Gly Thr Leu Thr Ile Asn Ala Lys Asp
1425 1430 1435 1440

Ala Lys Leu Asp Gly Ala Ala Ser Gly Asp Arg Thr Val Val Asn Ala
1445 1450 1455

Thr Asn Ala Ser Gly Ser Gly Asn Val Thr Ala Lys Thr Ser Ser Ser
1460 1465 1470

Val Asn Ile Thr Gly Asp Leu Asn Thr Ile Asn Gly Leu Asn Ile Ile
1475 1480 1485

Ser Glu Asn Gly Arg Asn Thr Val Arg Leu Arg Gly Lys Glu Ile Asp
1490 1495 1500

Val Lys Tyr Ile Gln Pro Gly Val Ala Ser Val Glu Glu Val Ile Glu
1505 1510 1515 1520

Ala Lys Arg Val Leu Glu Lys Val Lys Asp Leu Ser Asp Glu Glu Arg
1525 1530 1535

Glu Thr Leu Ala Lys Leu Gly Val Ser Ala Val Arg Phe Val Glu Pro
1540 1545 1550

Asn Asn Ala Ile Thr Val Asn Thr Gln Asn Glu Phe Thr Thr Lys Pro
1555 1560 1565

Ser Ser Gln Val Thr Ile Ser Glu Gly Lys Ala Cys Phe Ser Ser Gly
1570 1575 1580

Asn Gly Ala Arg Val Cys Thr Asn Val Ala Asp Asp Gly Gln Gln Pro
1585 1590 1595 1600

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Val Asp Glu Val Ile Glu Ala Lys Arg Ile Leu Glu Lys Val Lys Asp
1 5 10 15

Leu Ser Asp Glu Glu Arg Glu Ala Leu Ala Lys Leu Gly
20 25

CLAIMS

What I claim is:

1. An isolated and purified nucleic acid molecule encoding a high molecular weight protein (HMW) HMW3 or HMW4 of a non-typeable *Haemophilus* strain or a variant or fragment of said protein retaining the immunological ability to protect against disease caused by a non-typeable *Haemophilus* strain, having:

   (a) the DNA sequence shown in Figure 8 (SEQ ID No: 7) and encoding protein HMW3 having the derived amino acid sequence of Figure 10 (SEQ ID No: 9), or

   (b) the DNA sequence shown in Figure 9 (SEQ ID No: 8) and encoding protein HMW4 having the derived amino acid sequence of Figure 10 (SEQ ID No: 10).

2. An isolated and purified nucleic acid molecule encoding a high molecular weight protein (HMW) of a non-typeable *Haemophilus* strain, which is selected from the group consisting of:

   (a) a DNA sequence as shown in any one of Figures 8 and 9 (SEQ ID Nos: 7 and 8);

   (b) a DNA sequence encoding an amino acid sequence as shown in Figure 10 (SEQ ID Nos: 9 and 10); or

   (c) a DNA sequence encoding a high molecular weight protein of a non-typeable *Haemophilus* strain which hybridizes under stringent conditions to any one of the DNA sequences of (a) and (b).

3. The nucleic acid molecule of claim 2 wherein the DNA sequence (c) have at least about a 90% identity of sequence to the DNA sequences (a) or (b).

4. A vector for transformation of a host comprising the nucleic acid molecule of claim 2.

5. An isolated and purified high molecular weight (HMW) protein of non-typeable *Haemophilus* or any variant or fragment thereof retaining the immunological ability to protect against disease caused by a non-typeable *Haemophilus* strain, which is characterized by at least

one surface-exposed B-cell epitope which is recognized by monoclonal antibody AD6.

6. The protein of claim 5 which is HMW1 encoded by the DNA sequence shown in Figure 1 (SEQ ID No: 1), having the derived amino acid sequence of Figure 2 (SEQ ID No: 2) and having an apparent molecular weight of 125 kDa.

7. The protein claim 5 which is HMW2 encoded by the DNA sequence shown in Figure 3 (SEQ ID No: 3) and having the derived amino acid sequence of Figure 4 (SEQ ID No: 4) and having an apparent molecular weight of 120 kDa.

8. The protein claimed in claim 5 which is HMW3 encoded by the DNA sequence shown in Figure 8 (SEQ ID No: 7) and having the derived amino acid sequence of Figure 10 (SEQ ID No: 9) and having an apparent molecular weight of 125 kDa.

9. The protein claimed in claim 5 which is HMW4 encoded by the DNA sequence shown in Figure 9 (SEQ ID No: 8) and having the derived amino acid sequence shown in Figure 10 (SEQ ID No: 10) and having an apparent molecular weight of 123 kDa.

10. A conjugate comprising a protein as claimed in claim 5 linked to an antigen, hapten or polysaccharide for eliciting an immune response to said antigen, hapten or polysaccharide.

11. The conjugate as claimed in claim 10 wherein said polysaccharide is a protective polysaccharide against *Haemophilus influenzae* type b.

12. A synthetic peptide having an amino acid sequence containing at least six amino acids and no more than 150 amino acids and corresponding to at least one protective epitope of a high molecular weight protein HMW1, HMW2, HMW3 or HMW4 of non-typeable *Haemophilus influenzae*, wherein the epitope is recognized by at least one of monoclonal antibodies AD6 and 10C5.

13. The peptide as claimed in claim 12 wherein the epitope is located within 75 amino acids of the carboxy terminus of the HMW1 or HMW2 protein.

FIG. 1A. DNA SEQUENCE OF HIGH MOLECULAR WEIGHT PROTEIN I (HMW1)

```
  1 ACAGCGTTCT CTTAATACTA GTACAAACCC ACAATAAAAT ATGACAAACA
 51 ACAATTACAA CACCTTTTTT GCAGTCTATA TGCAAATATT TTAAAAAATA
101 GTATAAATCC GCCATATAAA ATGGTATAAT CTTTCATCTT TCATCTTTCA
151 TCTTTCATCT TTCATCTTTC ATCTTTCATC TTTCATCTTT CATCTTTCAT
201 CTTTCATCTT TCATCTTTCA TCTTTCATCT TTCATCTTTC ACATGCCCTG
251 ATGAACCGAG GGAAGGGAGG GAGGGGCAAG AATGAAGAGG GAGCTGAACG
301 AACGCAAATG ATAAAGTAAT TTAATTGTTC AACTAACCTT AGGAGAAAAT
351 ATGAACAAGC TATATCGTCT CAAATTCAGC AAACGCCTGA ATGCTTTGGT
401 TGCTGTGTCT GAATTGGCAC GGGGTTGTGA CCATTCCACA GAAAAAGGCA
451 GCGAAAAACC TGCTCGCATG AAAGTGCGTC ACTTAGCGTT AAAGCCACTT
501 TCCGCTATGT TACTATCTTT AGGTGTAACA TCTATTCCAC AATCTGTTTT
551 AGCAAGCGGC TTACAAGGAA TGGATGTAGT ACACGGCACA GCCACTATGC
601 AAGTAGATGG TAATAAAACC ATTATCCGCA ACAGTGTTGA CGATATCATT
651 AATTGGAAAC AATTTAACAT CGACCAAAAT GAAATGGTGC AGTTTTTACA
701 AGAAAACAAC AACTCCGCCG TATTCAACCG TGTTACATCT AACCAAATCT
```

FIG. 1B.

751	CCCAATTAAA	AGGGATTTTA	GATTCTAACG	GACAAGTCTT	TTTAATCAAC
801	CCAAATGGTA	TCACAATAGG	TAAAGACGCA	ATTATTAACA	CTAATGGCTT
851	TACGGCTTCT	ACGCTAGACA	TTTCTAACGA	AAACATCAAG	GCGCGTAATT
901	TCACCTTCGA	GCAAACCAAA	GATAAAGCGC	TCGCTGAAAT	TGTGAATCAC
951	GGTTTAATTA	CTGTCGGTAA	AGACGGCAGT	GTAAATCTTA	TTGGTGGCAA
1001	AGTGAAAAAC	GAGGGTGTGA	TTAGCGTAAA	TGGTGGCAGC	ATTTCTTTAC
1051	TCGCAGGGCA	AAAAATCACC	ATCAGCGATA	TAATAAACCC	AACCATTACT
1101	TACAGCATTG	CCGCGCCTGA	AAATGAAGCG	GTCAATCTGG	GCGATATTTT
1151	TGCCAAAGGC	GGTAACATTA	ATGTCCGTGC	TGCCACTATT	CGAAACCAAG
1201	GTAAACTTTC	TGCTGATTCT	GTAAGCAAAG	ATAAAAGCGG	CAATATTGTT
1251	CTTTCCGCCA	AAGAGGGTGA	AGCGGAAATT	GGCGGTGTAA	TTTCCGCTCA
1301	AAATCAGCAA	GCTAAAGGCG	GCAAGCTGAT	GATTACAGGC	GATAAAGTCA
1351	CATTAAAAAC	AGGTGCAGTT	ATCGACCTTT	CAGGTAAAGA	AGGGGGAGAA
1401	ACTTACCTTG	GCGGTGACGA	GCGCGGCGAA	GGTAAAAAGG	GCATTCAATT
1451	AGCAAAGAAA	ACCTCTTTAG	AAAAAGGCTC	AACCATCAAT	GTATCAGGCA
1501	AAGAAAAAGG	CGGACGCGCT	ATTGTGTGGG	GCGATATTGC	GTTAATTGAC

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FIG.1C.

1551	GGCAATATTA	ACGCTCAAGG	TAGTGGTGAT	ATCGCTAAAA	CCGGTGGTTT
1601	TGTGGAGACG	TCGGGGCATG	ATTTATTCAT	CAAAGACAAT	GCAATTGTTG
1651	ACGCCAAAGA	GTGGTTGTTA	GACCCGGATA	ATGTATCTAT	TAATGCAGAA
1701	ACAGCAGGAC	GCAGCAATAC	TTCAGAAGAC	GATGAATACA	CGGGATCCGG
1751	GAATAGTGCC	AGCACCCCAA	AACGAAACAA	AGAAAAGACA	ACATTAACAA
1801	ACACAACTCT	TGAGAGTATA	CTAAAAAAAG	GTACCTTTGT	TAACATCACT
1851	GCTAATCAAC	GCATCTATGT	CAATAGCTCC	ATTAATTTAT	CCAATGGCAG
1901	CTTAACTCTT	TGGAGTGAGG	GTCGGAGCGG	TGGCGGCGTT	GAGATTAACA
1951	ACGATATTAC	CACCGGTGAT	GATACCAGAG	GTGCAAACTT	AACAATTTAC
2001	TCAGGCGGCT	GGGTTGATGT	TCATAAAAAT	ATCTCACTCG	GGGCGCAAGG
2051	TAACATAAAC	ATTACAGCTA	AACAAGATAT	CGCCTTTGAG	AAAGGAAGCA
2101	ACCAAGTCAT	TACAGGTCAA	GGGACTATTA	CCTCAGGCAA	TCAAAAAGGT
2151	TTTAGATTTA	ATAATGTCTC	TCTAAACGGC	ACTGGCAGCG	GACTGCAATT
2201	CACCACTAAA	AGAACCAATA	AATACGCTAT	CACAAATAAA	TTTGAAGGGA
2251	CTTTAAATAT	TTCAGGGAAA	GTGAACATCT	CAATGGTTTT	ACCTAAAAAT
2301	GAAAGTGGAT	ATGATAAATT	CAAAGGACGC	ACTTACTGGA	ATTTAACCTC

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FIG.1D.

```
2351 CTTAAATGTT TCCGAGAGTG GCGAGTTTAA CCTCACTATT GACTCCAGAG
2401 GAAGCGATAG TGCAGGCACA CTTACCCAGC CTTATAATTT AAACGGTATA
2451 TCATTCAACA AAGACACTAC CTTTAATGTT GAACGAAATG CAAGAGTCAA
2501 CTTTGACATC AAGGCACCAA TAGGGATAAA TAAGTATTCT AGTTTGAATT
2551 ACGCATCATT TAATGGAAAC ATTTCAGTTT CGGGAGGGGG GAGTGTTGAT
2601 TTCACACTTC TCGCCTCATC CTCTAACGTC CAAACCCCCG GTGTAGTTAT
2651 AAATTCTAAA TACTTTAATG TTTCAACAGG GTCAAGTTTA AGATTTAAAA
2701 CTTCAGGCTC AACAAAAACT GGCTTCTCAA TAGAGAAAGA TTTAACTTTA
2751 AATGCCACCG GAGGCAACAT AACACTTTTG CAAGTTGAAG GCACCGATGG
2801 AATGATTGGT AAAGGCATTG TAGCCAAAAA AAACATAACC TTTGAAGGAG
2851 GTAACATCAC CTTTGGCTCC AGGAAAGCCG TAACAGAAAT CGAAGGCAAT
2901 GTTACTATCA ATAACAACGC TAACGTCACT CTTATCGGTT CGGATTTTGA
2951 CAACCATCAA AAACCTTTAA CTATTAAAAA AGATGTCATC ATTAATAGCG
3001 GCAACCTTAC CGCTGGAGGC AATATTGTCA ATATAGCCGG AAATCTTACC
3051 GTTGAAAGTA ACGCTAATTT CAAAGCTATC ACAAATTTCA CTTTTAATGT
3101 AGGCGGCTTG TTTGACAACA AAGGCAATTC AAATATTTCC ATTGCCAAAG
3151 GAGGGGCTCG CTTTAAAGAC ATTGATAATT CCAAGAATTT AAGCATCACC
```

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FIG. 1E.

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3201  ACCAACTCCA GCTCCACTTA CCGCACTATT ATAAGCGGCA ATATAACCAA
3251  TAAAAACGGT GATTTAAATA TTACGAACGA AGGTAGTGAT ACTGAAATGC
3301  AAATTGGCGG CGATGTCTCG CAAAAAGAAG GTAATCTCAC GATTTCTTCT
3351  GACAAAATCA ATATTACCAA ACAGATAACA ATCAAGGCAG GTGTTGATGG
3401  GGAGAATTCC GATTCAGACG CGACAAACAA TGCCAATCTA ACCATTAAAA
3451  CCAAAGAATT GAAATTAACG CAAGACCTAA ATATTTCAGG TTTCAATAAA
3501  GCAGAGATTA CAGCTAAAGA TGGTAGTGAT TTAACTATTG GTAACACCAA
3551  TAGTGCTGAT GGTACTAATG CCAAAAAAGT AACCTTTAAC CAGGTTAAAG
3601  ATTCAAAAAT CTCTGCTGAC GGTCACAAGG TGACACTACA CAGCAAAGTG
3651  GAAACATCCG GTAGTAATAA CAACACTGAA GATAGCAGTG ACAATAATGC
3701  CGGCTTAACT ATCGATGCAA AAAATGTAAC AGTAAACAAC AATATTACTT
3751  CTCACAAAGC AGTGAGCATC TCTGCGACAA GTGGAGAAAT TACCACTAAA
3801  ACAGGTACAA CCATTAACGC AACCACTGGT AACGTGGAGA TAACCGCTCA
3851  AACAGGTAGT ATCCTAGGTG GAATTGAGTC CAGCTCTGGC TCTGTAACAC
3901  TTACTGCAAC CGAGGGCGCT CTTGCTGTAA GCAATATTTC GGGCAACACC
3951  GTTACTGTTA CTGCAAATAG CGGTGCATTA ACCACTTTGG CAGGCTCTAC
```

FIG.1F.

```
4001  AATTAAAGGA ACCGAGAGTG TAACCACTTC AAGTCAATCA GGCGATATCG
4051  GCGGTACGAT TTCTGGTGGC ACAGTAGAGG TTAAAGCAAC CGAAAGTTTA
4101  ACCACTCAAT CCAATTCAAA AATTAAAGCA ACAACAGGCG AGGCTAACGT
4151  AACAAGTGCA ACAGGTACAA TTGGTGGTAC GATTTCCGGT AATACGGTAA
4201  ATGTTACGGC AAACGCTGGC GATTTAACAG TTGGGAATGG CGCAGAAATT
4251  AATGCGACAG AAGGAGCTGC AACCTTAACT ACATCATCGG GCAAATTAAC
4301  TACCGAAGCT AGTTCACACA TTACTTCAGC CAAGGGTCAG GTAAATCTTT
4351  CAGCTCAGGA TGGTAGCGTT GCAGGAAGTA TTAATGCCGC CAATGTGACA
4401  CTAAATACTA CAGGCACTTT AACTACCGTG AAGGGTTCAA ACATTAATGC
4451  AACCAGCGGT ACCTTGGTTA TTAACGCAAA AGACGCTGAG CTAAATGGCG
4501  CAGCATTGGG TAACCACACA GTGGTAAATG CAACCAACGC AAATGGCTCC
4551  GGCAGCGTAA TCGCGACAAC CTCAAGCAGA GTGAACATCA CTGGGGATTT
4601  AATCACAATA AATGGATTAA ATATCATTTC AAAAAACGGT ATAAACACCG
4651  TACTGTTAAA AGGCGTTAAA ATTGATGTGA AATACATTCA ACCGGGTATA
4701  GCAAGCGTAG ATGAAGTAAT TGAAGCGAAA CGCATCCTTG AGAAGGTAAA
4751  AGATTTATCT GATGAAGAAA GAGAAGCGTT AGCTAAACTT GGAGTAAGTG
4801  CTGTACGTTT TATTGAGCCA AATAATACAA TTACAGTCGA TACACAAAAT
```

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FIG. 1G.

```
4851  GAATTTGCAA CCAGACCATT AAGTCGAATA GTGATTTCTG AAGGCAGGGC
4901  GTGTTTCTCA AACAGTGATG GCGCGACGGT GTGCGTTAAT ATCGCTGATA
4951  ACGGGCGGTA GCGGTCAGTA ATTGACAAGG TAGATTTCAT CCTGCAATGA
5001  AGTCATTTTA TTTTCGTATT ATTTACTGTG TGGGTTAAAG TTCAGTACGG
5051  GCTTTACCCA TCTTGTAAAA AATTACGGAG AATACAATAA AGTATTTTTA
5101  ACAGGTTATT ATTATG
```

FIG. 2A. AMINO ACID SEQUENCE OF HIGH MOLECULAR WEIGHT PROTEIN I

```
  1 MNKIYRLKFS KRLNALVAVS ELARGCDHST EKGSEKPARM KVRHLALKPL
 51 SAMLLSLGVT SIPQSVLASG LQGMDVVHGT ATMQVDGNKT IIRNSVDAII
101 NWKQFNIDQN EMVQFLQENN NSAVFNRVTS NQISQLKGIL DSNGQVFLIN
151 PNGITIGKDA IINTNGFTAS TLDISNENIK ARNFTFEQTK DKALAEIVNH
201 GLITVGKDGS VNLIGGKVKN EGVISVNGGS ISLLAGQKIT ISDIINPTIT
251 YSIAAPENEA VNLGDIFAKG GNINVRAATI RNQGKLSADS VSKDKSGNIV
301 LSAKEGEAEI GGVISAQNQQ AKGGKLMITG DKVTLKTGAV IDLSGKEGGE
351 TYLGGDERGE GKNGIQLAKK TSLEKGSTIN VSGKEKGGRA IVWGDIALID
401 GNINAQGSGD IAKTGGFVET SGHDLFIKDN AIVDAKEWLL DFDNVSINAE
451 TAGRSNTSED DEYTGSGNSA STPKRNKEKT TLTNTTLESI LKKGTFVNIT
501 ANQRIYVNSS INLSNGSLTL WSEGRSGGGV EINNDITTGD DTRGANLTIY
551 SGGWVDVHKN ISLGAQGNIN ITAKQDIAFE KGSNQVITGQ GTITSGNQKG
601 FRFNNVSLNG TGSGLQFTTK RTNKYAITNK FEGTLNISGK VNISMVLPKN
651 ESGYDKFKGR TYWNLTSLNV SESGEFNLTI DSRGSDSAGT LTQPYNLNGI
701 SFNKDTTFNV ERNARVNFDI KAPIGINKYS SLNYASFNGN ISVSGGGSVD
```

FIG. 2B.

751	FTLLASSSNV	QTPGVVINSK	YFNVSTGSSL	RFKTSGSTKT	GFSIEKDLTL
801	NATGGNITLL	QVEGTDGMIG	KGIVAKKNIT	FEGGNITFGS	RKAVTEIEGN
851	VTINNNANVT	LIGSDFDNHQ	KPLTIKKDVI	INSGNLTAGG	NIVNIAGNLT
901	VESNANFKAI	TNFTFNVGGL	FDNKGNSNIS	IAKGGARFKD	IDNSKNLSIT
951	TNSSSTYRTI	ISGNITNKNG	DLNITNEGSD	TEMQIGGDVS	QKEGNLTISS
1001	DKINITKQIT	IKAGVDGENS	DSDATNNANL	TIKTKELKLT	QDLNISGFNK
1051	AEITAKDGSD	LTIGNTNSAD	GTNAKKVTFN	QVKDSKISAD	GHKVTLHSKV
1101	ETSGSNNNTE	DSSDNNAGLT	IDAKNVTVNN	NITSHKAVSI	SATSGEITTK
1151	TGTTINATTG	NVEITAQTGS	ILGGIESSSG	SVTLTATEGA	LAVSNISGNT
1201	VTVTANSGAL	TTLAGSTIKG	TESVTTSSQS	GDIGGTISGG	TVEVKATESL
1251	TTQSNSKIKA	TTGEANVTSA	TGTIGGTISG	NTVNVTANAG	DLTVGNGAEI
1301	NATEGAATLT	TSSGKLTTEA	SSHITSAKGQ	VNLSAQDGSV	AGSINAANVT
1351	LNTTGTLTTV	KGSNINATSG	TLVINAKDAE	LNGAALGNHT	VVNATNANGS
1401	GSVIATTSSR	VNITGDLITI	NGLNIISKNG	INTVLLKGVK	IDVKYIQPGI
1451	ASVDEVIEAK	RILEKVKDLS	DEEREALAKL	GVSAVRFIEP	NNTITVDTQN
1501	EFATRPLSRI	VISEGRACFS	NSDGATVCVN	IADNGR	

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FIG. 3A. DNA SEQUENCE OF HIGH MOLECULAR WEIGHT PROTEIN II (HMW2)

```
  1 TAAATATACA AGATAATAAA AATAAATCAA GATTTTTGTG ATGACAAACA
 51 ACAATTACAA CACCTTTTTT GCAGTCTATA TGCAAATATT TTAAAAAAAT
101 AGTATAAATC CGCCATATAA AATGGTATAA TCTTTCATCT TTCATCTTTA
151 ATCTTTCATC TTTCATCTTT CATCTTTCAT CTTTCATCTT TCATCTTTCA
201 TCTTTCATCT TTCATCTTTC ATCTTTCATC TTTCATCTTT CACATGAAAT
251 GATGAACCGA GGGAAGGGAG GGAGGGGCAA GAATGAAGAG GGAGCTGAAC
301 GAACGCAAAT GATAAAGTAA TTTAATTGTT CAACTAACCT TAGGAGAAAA
351 TATGAACAAG ATATATCGTC TCAAATTCAG CAAACGCCTG AATGCTTTGG
401 TTGCTGTGTC TGAATTGGCA CGGGGTTGTG ACCATTCCAC AGAAAAAGGC
451 TTCCGCTATG TTACTATCTT TAGGTGTAAC CACTTAGCGT TAAAGCCACT
501 TTCCGCTATG TTACTATCTT TAGGTGTAAC ATCTATTCCA CAATCTGTTT
551 TAGCAAGCGG CTTACAAGGA ATGGATGTAG TACACGGCAC AGCCACTATG
601 CAAGTAGATG GTAATAAAAC CATTATCCGC AACAGTGTTG ACGCTATCAT
651 TAATTGGAAA CAATTTAACA TCGACCAAAA TGAAATGGTG CAGTTTTTAC
701 AAGAAAACAA CAACTCCGCC GTATTCAACC GTGTTACATC TAACCAAATC
```

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FIG.3B.

```
751   TCCCAATTAA AAGGGATTTT AGATTCTAAC GGACAAGTCT TTTTAATCAA
801   CCCAAATGGT ATCACAATAG GTAAAGACGC AATTATTAAC ACTAATGGCT
851   TTACGGCTTC TACGCTAGAC ATTTCTAACG AAAACATCAA GGCGCGTAAT
901   TTCACCTTCG AGCAAACCAA AGATAAAGCG CTCGCTGAAA TTGTGAATCA
951   CGGTTTAATT ACTGTCGGTA AAGACGGCAG TGTAAATCTT ATTGGTGGCA
1001  AAGTGAAAAA CGAGGGTGTG ATTAGCGTAA ATGGTGGCAG CATTTCTTTA
1051  CTCGCAGGGC AAAAAATCAC CATCAGCGAT ATAATAAACC CAACCATTAC
1101  TTACAGCATT GCCGCGCCTG AAAATGAAGC GGTCAATCTG GGCGATATTT
1151  TTGCCAAAGG CGGTAACATT AATGTCCGTG CTGCCACTAT TCGAAACCAA
1201  GGTAAACTTT CTGCTGATTC TGTAAGCAAA GATAAAAGCG GCAATATTGT
1251  TCTTTCCGCC AAAGAGGGTG AAGCGGAAAT TGGCGGTGTA ATTTCCGCTC
1301  AAAATCAGCA AGCTAAAGGC GGCAAGCTGA TGATTACAGG CGATAAAGTC
1351  ACATTAAAAA CAGGTGCAGT TATCGACCTT TCAGGTAAAG AAGGGGGAGA
1401  AACTTACCTT GGCGGTGACG AGCGCGGCGA AGGTAAAAAC GGCATTCAAT
1451  TAGCAAAGAA AACCTCTTTA GAAAAAGGCT CAACCATCAA TGTATCAGGC
1501  AAAGAAAAAG GCGGACGCGC TATTGTGTGG GGCGATATTG CGTTAATTGA
```

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FIG.3C.

```
1551  CGGCAATATT AACGCTCAAG GTAGTGGTGA TATCGCTAAA ACCGGTGGTT
1601  TTGTGGAGAC ATCGGGGCAT TATTTATCCA TTGACAGCAA TGCAATTGTT
1651  AAAACAAAAG AGTGGTTGCT AGACCCTGAT GATGTAACAA TTGAAGCCGA
1701  AGACCCCCTT CGCAATAATA CCGGTATAAA TGATGAATTC CCAACAGGCA
1751  CCGGTGAAGC AAGCGACCCT AAAAAAAATA GCGAACTCAA AACAACGCTA
1801  ACCAATACAA CTATTTCAAATTATCTGAAA AACGCCTGGA CAATGAATAT
1851  AACGGCATCA AGAAAACTTA CCGTTAATAG CTCAATCAAC ATCGGAAGCA
1901  ACTCCCACTT AATTCTCCAT AGTAAAGGTC AGCGTGGCGG AGGCGTTCAG
1951  ATTGATGGAG ATATTACTTC TAAAGGCGGA AATTTAACCA TTTATTCTGG
2001  CGGATGGGTT GATGTTCATA AAAATATTAC GCTTGATCAG GGTTTTTTAA
2051  ATATTACCGC CGCTTCCGTA GCTTTTGAAG GTGGAAATAA CAAAGCACGC
2101  GACGCGGCAA ATGCTAAAAT TGTCGCCCAG GGCACTGTAA CCATTACAGG
2151  AGAGGGAAAA GATTTCAGGG CTAACAACGT ATCTTTAAAC GGAACGGGTA
2201  AAGGTCTGAA TATCATTTCA TCAGTGAATA ATTTAACCCA CAATCTTAGT
2251  GGCACAATTA ACATATCTGG GAATATAACA ATTAACCAAA CTACGAGAAA
2301  GAACACCTCG TATTGGCAAA CCAGCCATGA TTCGCACTGG AACGTCAGTG
2351  CTCTTAATCT AGAGACAGGC GCAAATTTTA CCTTTATTAA ATACATTTCA
```

FIG.3D.

2401	AGCAATAGCA	AAGGCTTAAC	AACACAGTAT	AGAAGCTCTG	CAGGGGTGAA
2451	TTTTAACGGC	GTAAATGGCA	ACATGTCATT	CAATCTCAAA	GAAGGAGCGA
2501	AAGTTAATTT	CAAATTAAAA	CCAAACGAGA	ACATGAACAC	AAGCAAACCT
2551	TTACCAATTC	GGTTTTTAGC	CAATATCACA	GCCACTGGTG	GGGGCTCTGT
2601	TTTTTTTGAT	ATATATGCCA	ACCATTCTGG	CAGAGGGGCT	GAGTTAAAAA
2651	TGAGTGAAAT	TAATATCTCT	AACGGCGCTA	ATTTTACCTT	AAATTCCCAT
2701	GTTCGCGGCG	ATGACGCTTT	TAAAATCAAC	AAAGACTTAA	CCATAAATGC
2751	AACCAATTCA	AATTTCAGCC	TCAGACAGAC	GAAAGATGAT	TTTTATGACG
2801	GGTACGCACG	CAATGCCATC	AATTCAACCT	ACAACATATC	CATTCTGGGC
2851	GGTAATGTCA	CCCTTGGTGG	ACAAAACTCA	AGCAGCAGCA	TTACGGGGAA
2901	TATTACTATC	GAGAAAGCAG	CAAATGTTAC	GCTAGAAGCC	AATAACGCCC
2951	CTAATCAGCA	AAACATAAGG	GATAGAGTTA	TAAAACTTGG	CAGCTTGCTC
3001	GTTAATGGGA	GTTTAAGTTT	AACTGGCGAA	AATGCAGATA	TTAAAGGCAA
3051	TCTCACTATT	TCAGAAAGCG	CCACTTTTAA	AGGAAAGACT	AGAGATACCC
3101	TAAATATCAC	CGGCAATTTT	ACCAATAATG	GCACTGCCGA	AATTAATATA
3151	ACACAAGGAG	TGGTAAAACT	TGGCAATGTT	ACCAATGATG	GTGATTTAAA

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FIG. 3E.

```
3201 CATTACCACT CACGCTAAAC GCAACCAAAG AAGCATCATC GGCGGAGATA
3251 TAATCAACAA AAAAGGAAGC TTAAATATTA CAGACAGTAA TAATGATGCT
3301 GAAATCCAAA TTGGCGGCAA TATCTCGCAA AAAGAAGGCA ACCTCACGAT
3351 TTCTTCCGAT AAAATTAATA TCACCAAACA GATAACAATC AAAAAGGGTA
3401 TTGATGGAGA GGACTCTAGT TCAGATGCGA CAAGTAATGC CAACCTAACT
3451 ATTAAAACCA AAGAATTGAA ATTGACAGAA GACCTAAGTA TTTCAGGTTT
3501 CAATAAAGCA GAGATTACAG CCAAAGATGG TAGAGATTTA ACTATTGGCA
3551 ACAGTAATGA CGGTAACAGC GGTGCCGAAG CCAAAACAGT AACTTTTAAC
3601 AATGTTAAAG ATTCAAAAAT CTCTGCTGAC GGTCACAATG TGACACTAAA
3651 TAGCAAAGTG AAAACATCTA GCAGCAATGG CGGACGTGAA AGCAATAGCG
3701 ACAACGATAC CGGCTTAACT ATTACTGCAA AAAATGTAGA AGTAAACAAA
3751 GATATTACTT CTCTCAAAAC AGTAAATATC ACCGCGTCGG AAAAGGTTAC
3801 CACCACAGCA GGCTCGACCA TTAACGCAAC AAATGGCAAA GCAAGTATTA
3851 CAACCAAAAC AGGTGATATC AGCGGTACGA TTTCCGGTAA CACGGTAAGT
3901 GTTAGCGCGA CTGGTGATTT AACCACTAAA TCCGGCTCAA AAATTGAAGC
3951 GAAATCGGGT GAGGCTAATG TAACAAGTGC AACAGGTACA ATTGGCGGTA
```

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FIG.3F.

```
4001 CAATTTCCGG TAATACGGTA AATGTTACGG CAAACGCTGG CGATTTAACA
4051 GTTGGGAATG GCGCAGAAAT TAATGCGACA GAAGGAGCTG CAACCTTAAC
4101 CGCAACAGGG AATACCTTGA CTACTGAAGC CGGTTCTAGC ATCACTTCAA
4151 CTAAGGGTCA GGTAGACCTC TTGGCTCAGA ATGGTAGCAT CGCAGGAAGC
4201 ATTAATGCTG CTAATGTGAC ATTAAATACT ACAGGCACCT TAACCACCGT
4251 GGCAGGCTCG GATATTAAAG CAACCAGCGG CACCTTGGTT ATTAACGCAA
4301 AAGATGCTAA GCTAAATGGT GATGCATCAG GTGATAGTAC AGAAGTGAAT
4351 GCAGTCAACG CAAGCGGCTC TGGTAGTGTG ACTGCGGCAA CCTCAAGCAG
4401 TGTGAATATC ACTGGGGATT TAAACACAGT AAATGGGTTA AATATCATTT
4451 CGAAAGATGG TAGAAACACT GTGCGCTTAA GAGGCAAGGA AATTGAGGTG
4501 AAATATATCC AGCCAGGTGT AGCAAGTGTA GAAGAAGTAA TTGAAGCGAA
4551 ACGCGTCCTT GAAAAAGTAA AAGATTTATC TGATGAAGAA AGAGAAACAT
4601 TAGCTAAACT TGGTGTAAGT GCTGTACGTT TTGTTGAGCC AAATAATACA
4651 ATTACAGTCA ATACACAAAA TGAATTTACA ACCAGACCGT CAAGTCAAGT
4701 GATAATTTCT GAAGGTAAGG CGTGTTTCTC AAGTGGTAAT GGCGCACGAG
4751 TATGTACCAA TGTTGCTGAC GATGGACAGC CGTAGTCAGT AATTGACAAG
4801 GTAGATTTCA TCCTGCAATG AAGTCATTTT ATTTTCGTAT TATTTACTGT
```

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FIG. 3G.

4851 GTGGGTTAAA GTTCAGTACG GGCTTTACCC ATCTTGTAAA AAATTACGGA
4901 GAATACAATA AAGTATTTTT AACAGGTTAT TATTATG

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FIG. 4A. AMINO ACID SEQUENCE OF HIGH MOLECULAR WEIGHT PROTEIN 2

```
  1 MNKIYRLKFS KRLNALVAVS ELARGCDHST EKGSEKPARM KVRHLALKPL
 51 SAMLLSLGVT SIPQSVLASG LQGMDVVHGT ATMQVDGNKT IIRNSVDAII
101 NWKQFNIDQN EMVQFLQENN NSAVFNRVTS NQISQLKGIL DSNGQVFLIN
151 PNGITIGKDA IINTNGFTAS TLDISNENIK ARNFTFEQTK DKALAEIVNH
201 GLITVGKDGS VNLIGGKVKN EGVISVNGGS ISLLAGQKIT ISDIINPTIT
251 YSIAAPENEA VNLGDIFAKG GNINVRAATI RNQGKLSADS VSKDKSGNIV
301 LSAKEGEAEI GGVISAQNQQ AKGGKLMITG DKVTLKTGAV IDLSGKEGGE
351 TYLGGDERGE GKNGIQLAKK TSLEKGSTIN VSGKEKGGRA IVWGDIALID
401 GNINAQGSGD IAKTGGFVET SGHDLFIKDN AIVDAKEWLL DFDNVSINAE
451 DPLRNNTGIN DEFPTGTGEA SDPKKNSELK TTLTNTTISN YLKNAWTMNI
501 TASRKLTVNS SINIGSNSHL ILHSKGQRGG GVQIDGDITS KGGNLTIYSG
551 GWVDVHKNIT LDQGFLNITA ASVAFEGGNN KARDAANAKI VAQGTVTITG
601 EGKDFRANNV SLNGTGKGLN IISSVNNLTH NLSGTINISG NITINQTTRK
651 NTSYWQTSHD SHWNVSALNL ETGANFTFIK YISSNSKGLT TQYRSSAGVN
701 FNGVNGNMSF NLKEGAKVNF KLKPNENMNT SKPLPIRFLA NITATGGGSV
```

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FIG. 4B.

```
751  FFDIYANHSG RGAELKMSEI NISNGANFTL NSHVRGDDAF KINKDLTINA
801  TNSNFSLRQT KDDFYDGYAR NAINSTYNIS ILGGNVTLGG QNSSSSITGN
851  ITIEKAANVT LEANNAPNQQ NIRDRVIKLG SLLVNGSLSL TGENADIKGN
901  LTISESATFK GKTRDTLNIT GNFTNNGTAE INITQGVVKL GNVTNDGDLN
951  ITTHAKRNQR SIIGGDIINK KGSLNITDSN NDAEIQIGGN ISQKEGNLTI
1001 SSDKINITKQ ITIKKGIDGE DSSSDATSNA NLTIKTKELK LTEDLSISGF
1051 NKAEITAKDG RDLTIGNSND GNSGAEAKTV TFNNVKDSKI SADGHNVTLN
1101 SKVKTSSSNG GRESNSDNDT GLTITAKNVE VNKDITSLKT VNITASEKVT
1151 TTAGSTINAT NGKASITTKT GDISGTISGN TVSVSATVDL TTKSGSKIEA
1201 KSGEANVTSA TGTIGGTISG NTVNVTANAG DLTVGNGAEI NATEGAATLT
1251 ATGNTLTTEA GSSITSTKGQ VDLLAQNGSI AGSINAANVT LNTTGTLTTV
1301 AGSDIKATSG TLVINAKDAK LNGDASGDST EVNAVNASGS GSVTAATSSS
1351 VNITGDLNTV NGLNIISKDG RNTVRLRGKE IEVKYIQPGV ASVEEVIEAK
1401 RVLEKVKDLS DEERETLAKL GVSAVRFVEP NNTITVNTQN EFTTRPSSQV
1451 IISEGKACFS SGNGARVCTN VADDGQP
```

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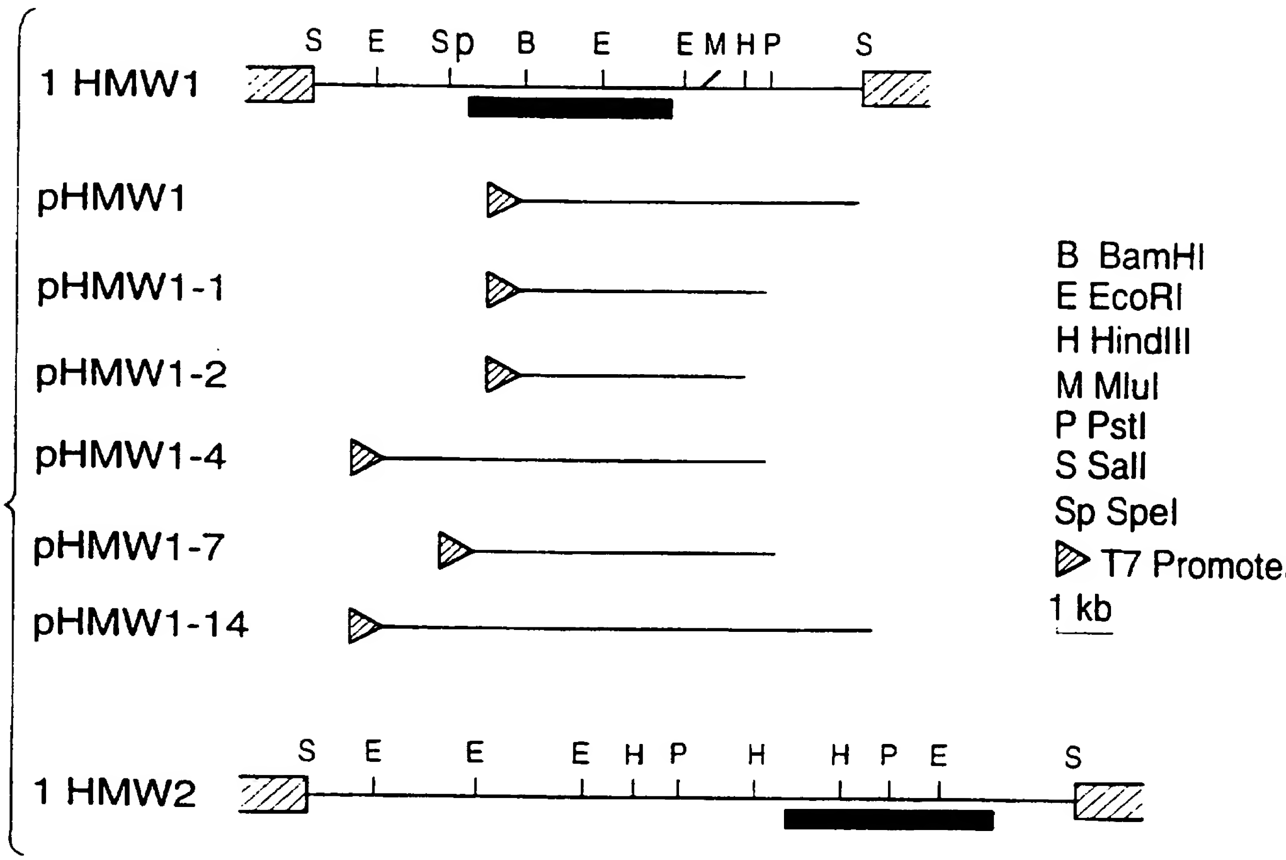
1 HMW1
S
E
Sp
B
E
E
M
H
P
S
pHMW1
pHMW1-1
pHMW1-2
pHMW1-4
pHMW1-7
pHMW1-14
1 HMW2
S
E
E
E
H
P
H
H
P
E
S
B BamHI
E EcoRI
H HindIII
M MluI
P PstI
S SalI
Sp SpeI
T7 Promoter
1 kb

FIG.5A.

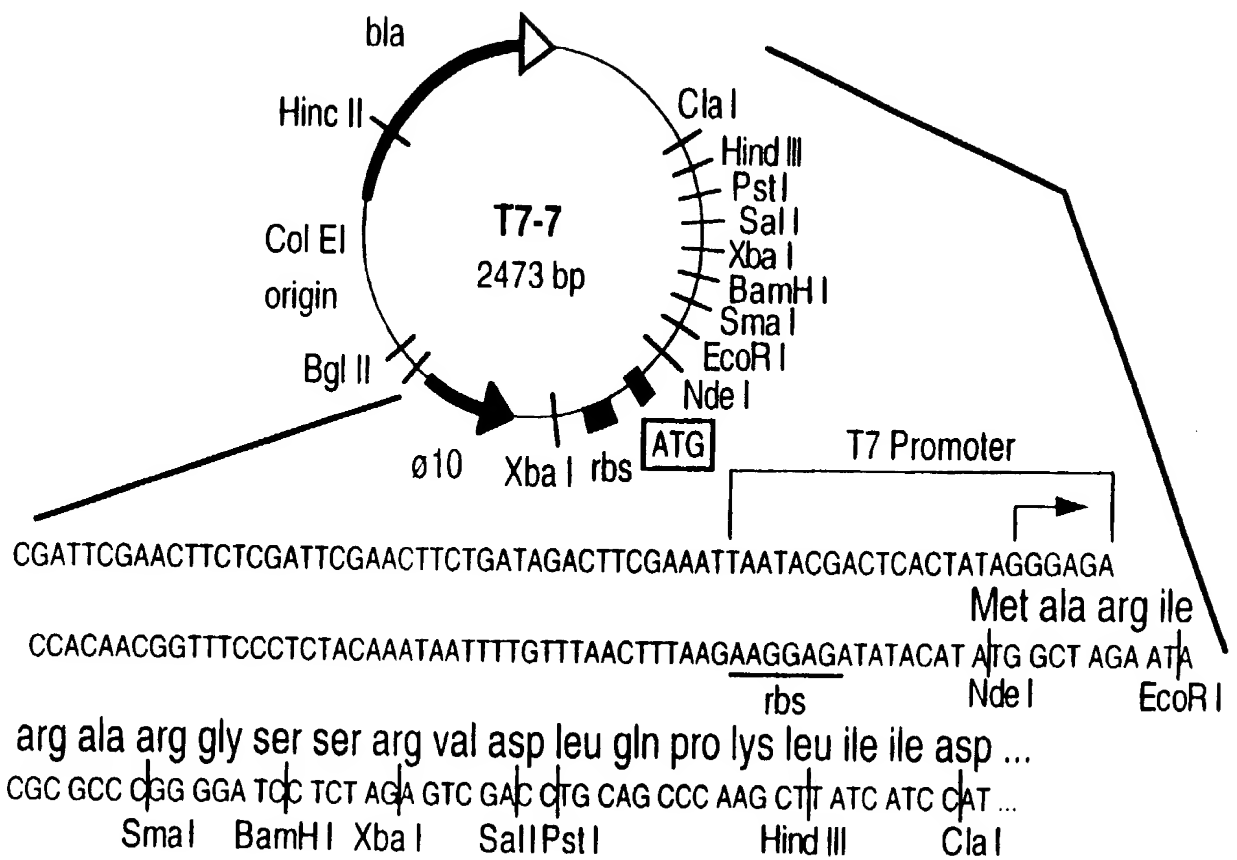

FIG.5B.

(A) Partial restriction maps of representative HMW1 and HMW2 recombinant phage and of HMW1 plasmid subclones. The shaded boxes indicate the locations of the structural genes. In the recombinant phage, transcription proceeds from left to right for the HMW1 gene and from right to left for the HMW2 gene. The methods used for construction of the plasmids shown are described in the text. (B) Restriction map of the T7 expression vector pT7-7. This vector contains the T7 RNA polymerase promoter ϕ10, a ribosome - binding site (rbs), and the translational start site for the T7 gene 10 protein upstream from a multiple cloning site (37).

FIG. 6A.

```
  1 ACAGCGTTCT CTTAATACTA GTACAAACCC ACAATAAAAT ATGACAAACA
 51 ACAATTACAA CACCTTTTTT GCAGTCTATA TGCAAATATT TTAAAAAATA
101 GTATAAATCC GCCATATAAA ATGGTATAAT CTTTCATCTT TCATCTTTCA
151 TCTTTCATCT TTCATCTTTC ATCTTTCATC TTTCATCTTT CATCTTTCAT
201 CTTTCATCTT TCATCTTTCA TCTTTCATCT TTCATCTTTC ACATGAAATG
251 ATGAACCGAG GGAAGGGAGG GAGGGGCAAG AATGAAGAGG GAGCTGAACG
301 AACGCAAATG ATAAAGTAAT TTAATTGTTC AACTAACCTT AGGAGAAAAT
351 ATGAACAAGA TATATCGTCT CAAATTCAGC AAACGCCTGA ATGCTTTGGT
401 TGCTGTGTCT GAATTGGCAC GGGGTTGTGA CCATTCCACA GAAAAAGGCA
451 GCGAAAAACC TGCTCGCATG AAAGTGCGTC ACTTAGCGTT AAAGCCACTT
501 TCCGCTATGT TACTATCTTT AGGTGTAACA TCTATTCCAC AATCTGTTTT
551 AGCAAGCGGC TTACAAGGAA TGGATGTAGT ACACGGCACA GCCACTATGC
601 AAGTAGATGG TAATAAAACC ATTATCCGCA ACAGTGTTGA CGCTATCATT
651 AATTGGAAAC AATTTAACAT CGACCAAAAT GAAATGGTGC AGTTTTTACA
701 AGAAAACAAC AACTCCGCCG TATTCAACCG TGTTACATCT AACCAAATCT
751 CCCAATTAAA AGGGATTTTA GATTCTAACG GACAAGTCTT TTTAATCAAC
```

FIG.6B.

801 CCAAATGGTA TCACAATAGG TAAAGACGCA ATTATTAACA CTAATGGCTT
851 TACGGCTTCT ACGCTAGACA TTTCTAACGA AAACATCAAG GCGCGTAATT
901 TCACCTTCGA GCAAACCAAA GATAAAGCGC TCGCTGAAAT TGTGAATCAC
951 GGTTTAATTA CTGTCGGTAA AGACGGCAGT GTAAATCTTA TTGGTGGCAA
1001 AGTGAAAAAC GAGGGTGTGA TTAGCGTAAA TGGTGGCAGC ATTTCTTTAC
1051 TCGCAGGGCA AAAAATCACC ATCAGCGATA TAATAAACCC AACCATTACT
1101 TACAGCATTG CCGCGCCTGA AAATGAAGCG GTCAATCTGG GCGATATTTT
1151 TGCCAAAGGC GGTAACATTA ATGTCCGTGC TGCCACTATT CGAAACCAAG
1251 CTTTCCGCCA AAGAGGGTGA AGCGGAAATT GGCGGTGTAA TTTCCGCTCA
1301 AAATCAGCAA GCTAAAGGCG GCAAGCTGAT GATTACAGGC GATAAAGTCA
1351 CATTAAAAAC AGGTGCAGTT ATCGACCTTT CAGGTAAAGA AGGGGGAGAA
1401 ACTTACCTTG GCGGTGACGA GCGCGGCGAA GGTAAAAACG GCATTCAATT
1451 AGCAAAGAAA ACCTCTTTAG AAAAAGGCTC AACCATCAAT GTATCAGGCA
1501 AAGAAAAAGG CGGACGCGCT ATTGTGTGGG GCGATATTGC GTTAATTGAC
1551 GGCAATATTA ACGCTCAAGG TAGTGGTGAT ATCGCTAAAA CCGGTGGTTT
1601 TGTGGAGACG TCGGGGCATG ATTTATTCAT CAAAGACAAT GCAATTGTTG

FIG.6C.

1651	ACGCCAAAGA	GTGGTTGTTA	GACCCGGATA	ATGTATCTAT	TAATGCAGAA
1701	ACAGCAGGAC	GCAGCAATAC	TTCAGAAGAC	GATGAATACA	CGGGATCCGG
1751	GAATAGTGCC	AGCACCCCAA	AACGAAACAA	AGAAAAGACA	ACATTAACAA
1801	ACACAACTCT	TGAGAGTATA	CTAAAAAAAG	GTACCTTTGT	TAACATCACT
1851	GCTAATCAAC	GCATCTATGT	CAATAGCTCC	ATTAATTTAT	CCAATGGCAG
1901	CTTAACTCTT	TGGAGTGAGG	GTCGGAGCGG	TGGCGGCGTT	GAGATTAACA
1951	ACGATATTAC	CACCGGTGAT	GATACCAGAG	GTGCAAACTT	AACAATTTAC
2001	TCAGGCGGCT	GGGTTGATGT	TCATAAAAAT	ATCTCACTCG	GGGCGCAAGG
2051	TAACATAAAC	ATTACAGCTA	AACAAGATAT	CGCCTTTGAG	AAAGGAAGCA
2101	ACCAAGTCAT	TACAGGTCAA	GGGACTATTA	CCTCAGGCAA	TCAAAAAGGT
2151	TTTAGATTTA	ATAATGTCTC	TCTAAACGGC	ACTGGCAGCG	GACTGCAATT
2201	CACCACTAAA	AGAACCAATA	AATACGCTAT	CACAAATAAA	TTTGAAGGGA
2251	CTTTAAATAT	TTCAGGGAAA	GTGAACATCT	CAATGGTTTT	ACCTAAAAAT
2301	GAAAGTGGAT	ATGATAAATT	CAAAGGACGC	ACTTACTGGA	ATTTAACCTC
2351	GAAAGTGGAT	ATGATAAATT	CAAAGGACGC	CCTCACTATT	GACTCCAGAG
2401	GAAGCGATAG	TGCAGGCACA	CTTACCCAGC	CTTATAATTT	AAACGGTATA
2451	TCATTCAACA	AAGACACTAC	CTTTAATGTT	GAACGAAATG	CAAGAGTCAA

FIG. 6D.

2501 CTTTGACATC AAGGCACCAA TAGGGATAAA TAAGTATTCT AGTTTGAATT
2551 ACGCATCATT TAATGGAAAC ATTTCAGTTT CGGGAGGGGG GAGTGTTGAT
2601 TTCACACTTC TCGCCTCATC CTCTAACGTC CAAACCCCCG GTGTAGTTAT
2651 AAATTCTAAA TACTTTAATG TTTCAACAGG GTCAAGTTTA AGATTTAAAA
2701 CTTCAGGCTC AACAAAAACT GGCTTCTCAA TAGAGAAAGA TTTAACTTTA
2751 AATGCCACCG GAGGCAACAT AACACTTTTG CAAGTTGAAG GCACCGATGG
2801 AATGATTGGT AAAGGCATTG TAGCCAAAAA AAACATAACC TTTGAAGGAG
2851 GTAAGATGAG GTTTGGCTCC AGGAAAGCCG TAACAGAAAT CGAAGGCAAT
2901 GTTACTATCA ATAACAACGC TAACGTCACT CTTATCGGTT CGGATTTTGA
2951 CAACCATCAA AAACCTTTAA CTATTAAAAA AGATGTCATC ATTAATAGCG
3001 GCAACCTTAC CGCTGGAGGC AATATTGTCA ATATAGCCGG AAATCTTACC
3051 GTTGAAAGTA ACGCTAATTT CAAAGCTATC ACAAATTTCA CTTTTAATGT
3101 AGGCGGCTTG TTTGACAACA AAGGCAATTC AAATATTTCC ATTGCCAAAG
3151 GAGGGGCTCG CTTTAAAGAC ATTGATAATT CCAAGAATTT AAGCATCACC
3201 ACCAACTCCA GCTCCACTTA CCGCACTATT ATAAGCGGCA ATATAACCAA
3251 TAAAAACGGT GATTTAAATA TTACGAACGA AGGTAGTGAT ACTGAAATGC

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FIG. 6E.

```
3301  AAATTGGCGG CGATGTCTCG CAAAAAGAAG GTAATCTCAC GATTTCTTCT
3351  GACAAAATCA ATATTACCAA ACAGATAACA ATCAAGGCAG GTGTTGATGG
3401  GGAGAATTCC GATTCAGACG CGACAAACAA TGCCAATCTA ACCATTAAAA
3451  CCAAAGAATT GAAATTAACG CAAGACCTAA ATATTTCAGG TTTCAATAAA
3501  GCAGAGATTA CAGCTAAAGA TGGTAGTGAT TTAACTATTG GTAACACCAA
3551  TAGTGCTGAT GGTACTAATG CCAAAAAAGT AACCTTTAAC CAGGTTAAAG
3601  ATTCAAAAAT CTCTGCTGAC GGTCACAAGG TGACACTACA CAGCAAAGTG
3651  GAAACATCCG GTAGTAATAA CAACACTGAA GATAGCAGTG ACAATAATGC
3701  CGGCTTAACT ATCGATGCAA AAAATGTAAC AGTAAACAAC AATATTACTT
3751  CTCACAAAGC AGTGAGCATC TCTGCGACAA GTGGAGAAAT TACCACTAAA
3801  ACAGGTACAA CCATTAACGC AACCACTGGT AACGTGGAGA TAACCGCTCA
3851  AACAGGTAGT ATCCTAGGTG GAATTGAGTC CAGCTCTGGC TCTGTAACAC
3901  TTACTGCAAC CGAGGGCGCT CTTGCTGTAA GCAATATTTC GGGCAACACC
3951  GTTACTGTTA CTGCAAATAG CGGTGCATTA ACCACTTTGG CAGGCTCTAC
4001  AATTAAAGGA ACCGAGAGTG TAACCACTTC AAGTCAATCA GGCGATATCG
4051  GCGGTACGAT TTCTGGTGGC ACAGTAGAGG TTAAAGCAAC CGAAAGTTTA
```

FIG.6F.

```
4101 ACCACTCAAT CCAATTCAAA AATTAAAGCA ACAACAGGCG AGGCTAACGT
4151 AACAAGTGCA ACAGGTACAA TTGGTGGTAC GATTTCCGGT AATACGGTAA
4201 ATGTTACGGC AAACGCTGGC GATTTAACAG TTGGGAATGG CGCAGAAATT
4251 AATGCGACAG AAGGAGCTGC AACCTTAACT ACATCATCGG GCAAATTAAC
4301 TACCGAAGCT AGTTCACACA TTACTTCAGC CAAGGGTCAG GTAAATCTTT
4351 CAGCTCAGGA TGGTAGCGTT GCAGGAAGTA TTAATGCCGC CAATGTGACA
4401 CTAAATACTA CAGGCACTTT AACTACCGTG AAGGGTTCAA ACATTAATGC
4451 AACCAGCGGT ACCTTGGTTA TTAACGCAAA AGACGCTGAG CTAAATGGCG
4501 CAGCATTGGG TAACCACACA GTGGTAAATG CAACCAACGC AAATGGCTCC
4551 GGCAGCGTAA TCGCGACAAC CTCAAGCAGA GTGAACATCA CTGGGGATTT
4601 AATCACAATA AATGGATTAA ATATCATTTC AAAAAACGGT ATAAACACCG
4651 TACTGTTAAA AGGCGTTAAA ATTGATGTGA AATACATTCA ACCGGGTATA
4701 GCAAGCGTAG ATGAAGTAAT TGAAGCGAAA CGCATCCTTG AGAAGGTAAA
4751 AGATTTATCT GATGAAGAAA GAGAAGCGTT AGCTAAACTT GGCGTAAGTG
4801 CTGTACGTTT TATTGAGCCA AATAATACAA TTACAGTCGA TACACAAAAT
4851 GAATTTGCAA CCAGACCATT AAGTCGAATA GTGATTTCTG AAGGCAGGGC
4901 GTGTTTCTCA AACAGTGATG GCGCGACGGT GTGCGTTAAT ATCGCTGATA
```

FIG.6G.

```
4951 ACGGGCGGTA GCGGTCAGTA ATTGACAAGG TAGATTTCAT CCTGCAATGA
5001 AGTCATTTTA TTTTCGTATT ATTTACTGTG TGGGTTAAAG TTCAGTACGG
5051 GCTTTACCCA TCTTGTAAAA AATTACGGAG AATACAATAA AGTATTTTTA
5101 ACAGGTTATT ATTATGAAAA ATATAAAAAG CAGATTAAAA CTCAGTGCAA
5151 TATCAGTATT GCTTGGCCTG GCTTCTTCAT CATTGTATGC AGAAGAAGCG
5201 TTTTTAGTAA AAGGCTTTCA GTTATCTGGT GCACTTGAAA CTTTAAGTGA
5251 AGACGCCCAA CTGTCTGTAG CAAAATCTTT ATCTAAATAC CAAGGCTCGC
5301 AAACTTTAAC AAACCTAAAA ACAGCACAGC TTGAATTACA GGCTGTGCTA
5351 GATAAGATTG AGCCAAATAA GTTTGATGTG ATATTGCCAC AACAAACCAT
5401 TACGGATGGC AATATTATGT TTGAGCTAGT CTCGAAATCA GCCGCAGAAA
5451 GCCAAGTTTT TTATAAGGCG AGCCAGGGTT ATAGTGAAGA AAATATCGCT
5501 CGTAGCCTGC CATCTTTGAA ACAAGGAAAA GTGTATGAAG ATGGTCGTCA
5551 GTGGTTCGAT TTGCGTGAAT TCAATATGGC AAAAGAAAAT CCACTTAAAG
5601 TCACTCGCGT GCATTACGAG TTAAACCCTA AAAACAAAAC CTCTGATTTG
5651 GTAGTTGCAG GTTTTTCGCC TTTTGGCAAA ACGCGTAGCT TTGTTTCCTA
5701 TGATAATTTC GGCGCAAGGG AGTTTAACTA TCAACGTGTA AGTCTAGGTT
```

FIG. 6H.

```
5751 TTGTAAATGC CAATTTGACC GGACATGATG ATGTATTAAA TCTAAACGCA
5801 TTGACCAATG TAAAAGCACC ATCAAAATCT TATGCGGTAG GCATAGGATA
5851 TACTTATCCG TTTTATGATA AACACCAATC CTTAAGTCTT TATACCAGCA
5901 TGAGTTATGC TGATTCTAAT GATATCGACG GCTTACCAAG TGCGATTAAT
5951 CGTAAATTAT CAAAAGGTCA ATCTATCTCT GCGAATCTGA AATGGAGTTA
6001 TTATCTCCCG ACATTTAACC TTGGAATGGA AGACCAGTTT AAAATTAATT
6051 TAGGCTACAA CTACCGCCAT ATTAATCAAA CATCCGAGTT AAACACCCTG
6101 GGTGCAACGA AGAAAAAATT TGCAGTATCA GGCGTAAGTG CAGGCATTGA
6151 TGGACATATC CAATTTACCC CTAAAACAAT CTTTAATATT GATTTAACTC
6201 ATCATTATTA CGCGAGTAAA TTACCAGGCT CTTTTGGAAT GGAGCGCATT
6251 GGCGAAACAT TTAATCGCAG CTATCACATT AGCACAGCCA GTTTAGGGTT
6301 GAGTCAAGAG TTTGCTCAAG GTTGGCATTT TAGCAGTCAA TTATCGGGTC
6351 AGTTTACTCT ACAAGATATA AGTAGCATAG ATTTATTCTC TGTAACAGGT
6401 ACTTATGGCG TCAGAGGCTT TAAATACGGC GGTGCAAGTG GTGAGCGCGG
6451 TCTTGTATGG CGTAATGAAT TAAGTATGCC AAAATACACC CGCTTTCAAA
6501 TCAGCCCTTA TGCGTTTTAT GATGCAGGTC AGTTCCGTTA TAATAGCGAA
6551 AATGCTAAAA CTTACGGCGA AGATATGCAC ACGGTATCCT CTGCGGGTTT
```

FIG. 6I.

```
6601  AGGCATTAAA ACCTCTCCTA CACAAAACTT AAGCTTAGAT GCTTTTGTTG
6651  CTCGTCGCTT TGCAAATGCC AATAGTGACA ATTTGAATGG CAACAAAAAA
6701  CGCACAAGCT CACCTACAAC CTTCTGGGGT AGATTAACAT TCAGTTTCTA
6751  ACCCTGAAAT TTAATCAACT GGTAAGCGTT CCGCCTACCA GTTTATAACT
6801  ATATGCTTTA CCCGCCAATT TACAGTCTAT ACGCAACCCT GTTTTCATCC
6851  TTATATATCA AACAAACTAA GCAAACCAAG CAAACCAAGC AAACCAAGCA
6901  AACCAAGCAA ACCAAGCAAA CCAAGCAAAC CAAGCAAACC AAGCAAACCA
6951  AGCAAACCAA GCAAACCAAG CAAACCAAGC AAACCAAGCA ATGCTAAAAA
7001  ACAATTTATA TGATAAACTA AAACATACTC CATACCATGG CAATACAAGG
7051  GATTTAATAA TATGACAAAA GAAAATTTAC AAAGTGTTCC ACAAAATACG
7101  ACCGCTTCAC TTGTAGAATC AAACAACGAC CAAACTTCCC TGCAAATACT
7151  TAAACAACCA CCCAAACCCA ACCTATTACG CCTGGAACAA CATGTCGCCA
7201  AAAAAGATTA TGAGCTTGCT TGCCGCGAAT TAATGGCGAT TTTGGAAAAA
7251  ATGGACGCTA ATTTTGGAGG CGTTCACGAT ATTGAATTTG ACGCACCTGC
7301  TCAGCTGGCA TATCTACCCG AAAAACTACT AATTCATTTT GCCACTCGTC
7351  TCGCTAATGC AATTACAACA CTCTTTTCCG ACCCCGAATT GGCAATTTCC
```

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FIG. 6J.

7401	GAAGAAGGGG	CATTAAAGAT	GATTAGCCTG	CAACGCTGGT	TGACGCTGAT
7451	TTTTGCCTCT	TCCCCCTACG	TTAACGCAGA	CCATATTCTC	AATAAATATA
7501	ATATCAACCC	AGATTCCGAA	GGTGGCTTTC	ATTTAGCAAC	AGACAACTCT
7551	TCTATTGCTA	AATTCTGTAT	TTTTTACTTA	CCCGAATCCA	ATGTCAATAT
7601	GAGTTTAGAT	GCGTTATGGG	CAGGGAATCA	ACAACTTTGT	GCTTCATTGT
7651	GTTTTGCGTT	GCAGTCTTCA	CGTTTTATTG	GTACTGCATC	TGCGTTTCAT
7701	AAAAGAGCGG	TGGTTTTACA	GTGGTTTCCT	AAAAAACTCG	CCGAAATTGC
7751	TAATTTAGAT	GAATTGCCTG	CAAATATCCT	TCATGATGTA	TATATGCACT
7801	GCAGTTATGA	TTTAGCAAAA	AACAAGCACG	ATGTTAAGCG	TCCATTAAAC
7851	GAACTTGTCC	GCAAGCATAT	CCTCACGCAA	GGATGGCAAG	ACCGCTACCT
7901	TTACACCTTA	GGTAAAAAGG	ACGGCAAACC	TGTGATGATG	GTACTGCTTG
7951	AACATTTTAA	TTCGGGACAT	TCGATTTATC	GCACGCATTC	AACTTCAATG
8001	ATTGCTGCTC	GAGAAAAATT	CTATTTAGTC	GGCTTAGGCC	ATGAGGGCGT
8051	TGATAACATA	GGTCGAGAAG	TGTTTGACGA	GTTCTTTGAA	ATCAGTAGCA
8101	ATAATATAAT	GGAGAGACTG	TTTTTTATCC	GTAAACAGTG	CGAAACTTTC
8151	CAACCCGCAG	TGTTCTATAT	GCCAAGCATT	GGCATGGATA	TTACCACGAT

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FIG. 6K.

```
8201  TTTTGTGAGC AACACTCGGC TTGCCCCTAT TCAAGCTGTA GCCTTGGGTC
8251  ATCCTGCCAC TACGCATTCT GAATTTATTG ATTATGTCAT CGTAGAAGAT
8301  GATTATGTGG GCAGTGAAGA TTGTTTAGC  GAAACCCTTT TACGCTTACC
8351  CAAAGATGCC CTACCTTATG TACCATCTGC ACTCGCCCCA CAAAAAGTGG
8401  ATTATGTACT CAGGGAAAAC CCTGAAGTAG TCAATATCGG TATTGCCGCT
8451  ACCACAATGA AATTAAACCC TGAATTTTTG CTAACATTGC AAGAAATCAG
8501  AGATAAAGCT AAAGTCAAAA TACATTTTCA TTTCGCACTT GGACAATCAA
8551  CAGGCTTGAC ACACCCTTAT GTCAAATGGT TTATCGAAAG CTATTTAGGT
8601  GACGATGCCA CTGCACATCC CCACGCACCT TATCACGATT ATCTGGCAAT
8651  ATTGCGTGAT TGCGATATGC TACTAAATCC GTTTCCTTTC GGTAATACTA
8701  ACGGCATAAT TGATATGGTT ACATTAGGTT TAGTTGGTGT ATGCAAAACG
8751  GGGGATGAAG TACATGAACA TATTGATGAA GGTCTGTTTA AACGCTTAGG
8801  ACTACCAGAA TGGCTGATAG CCGACACACG AGAAACATAT ATTGAATGTG
8851  CTTTGCGTCT AGCAGAAAAC CATCAAGAAC GCCTTGAACT CCGTCGTTAC
8901  ATCATAGAAA ACAACGGCTT ACAAAAGCTT TTTACAGGCG ACCCTCGTCC
8951  ATTGGGCAAA ATACTGCTTA AGAAAACAAA TGAATGGAAG CGGAAGCACT
9001  TGAGTAAAAA ATAACGGTTT TTTAAAGTAA AAGTGCGGTT AATTTTCAAA
```

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FIG. 6L.

9051 GCGTTTTAAA AACCTCTCAA AAATCAACCG CACTTTTATC TTTATAACGC
9101 TCCCGCGCGC TGACAGTTTA TCTCTTTCTT AAAATACCCA TAAAATTGTG
9151 GCAATAGTTG GGTAATCAAA TTCAATTGTT GATACGGCAA ACTAAAGACG
9201 GCGCGTTCTT CGGCAGTCAT C

FIG. 7A.

1	CGCCACTTCA	ATTTTGGATT	GTTGAAATTC	AACTAACCAA	AAAGTGCGGT
51	TAAAATCTGT	GGAGAAAATA	GGTTGTAGTG	AAGAACGAGG	TAATTGTTCA
101	AAAGGATAAA	GCTCTCTTAA	TTGGGCATTG	GTTGGCGTTT	CTTTTTCGGT
151	TAATAGTAAA	TTATATTCTG	GACGACTATG	CAATCCACCA	ACAACTTTAC
201	CGTTGGTTTT	AAGCGTTAAT	GTAAGTTCTT	GCTCTTCTTG	GCGAATACGT
251	AATCCCATTT	TTTGTTTAGC	AAGAAAATGA	TCGGGATAAT	CATAATAGGT
301	GTTGCCCAAA	AATAAATTTT	GATGTTCTAA	AATCATAAAT	TTTGCAAGAT
351	ATTGTGGCAA	TTCAATACCT	ATTTGTGGCG	AAATCGCCAA	TTTTAATTCA
401	ATTTCTTGTA	GCATAATATT	TCCCACTCAA	ATCAACTGGT	TAAATATACA
451	AGATAATAAA	AATAAATCAA	GATTTTTGTG	ATGACAAACA	ACAATTACAA
501	CACCTTTTTT	GCAGTCTATA	TGCAAATATT	TTAAAAAAAT	AGTATAAATC
551	CGCCATATAA	AATGGTATAA	TCTTTCATCT	TTCATCTTTC	ATCTTTCATC
601	TTTCATCTTT	CATCTTTCAT	CTTTCATCTT	TCATCTTTCA	TCTTTCATCT
651	TTCATCTTTC	ATCTTTCATC	TTTCATCTTT	CACATGAAAT	GATGAACCGA
701	GGGAAGGGAG	GGAGGGGCAA	GAATGAAGAG	GGAGCTGAAC	GAACGCAAAT
751	GATAAAGTAA	TTTAATTGTT	CAACTAACCT	TAGGAGAAAA	TATGAACAAG

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FIG.7B.

801	ATATATCGTC	TCAAATTCAG	CAAACGCCTG	AATGCTTTGG	TTGCTGTGTC
851	TGAATTGGCA	CGGGGTTGTG	ACCATTCCAC	AGAAAAAGGC	AGCGAAAAAC
901	CTGCTCGCAT	GAAAGTGCGT	CACTTAGCGT	TAAAGCCACT	TTCCGCTATG
951	TTACTATCTT	TAGGTGTAAC	ATCTATTCCA	CAATCTGTTT	TAGCAAGCGG
1001	CAATTTAACA	TCGACCAAAA	TGAAATGGTG	CAGTTTTTAC	AAGAAAACAA
1051	GTAATAAAAC	CATTATCCGC	AACAGTGTTG	ACGCTATCAT	TAATTGGAAA
1101	CAATTTAACA	TCGACCAAAA	TGAAATGGTG	CAGTTTTTAC	AAGAAAACAA
1151	CAACTCCGCC	GTATTCAACC	GTGTTACATC	TAACCAAATC	TCCCAATTAA
1201	AAGGGATTTT	AGATTCTAAC	GGACAAGTCT	TTTTAATCAA	CCCAAATGGT
1251	ATCACAATAG	GTAAAGACGC	AATTATTAAC	ACTAATGGCT	TTACGGCTTC
1301	TACGCTAGAC	ATTTCTAACG	AAAACATCAA	GGCGCGTAAT	TTCACCTTCG
1351	AGCAAACCAA	AGATAAAGCG	CTCGCTGAAA	TTGTGAATCA	CGGTTTAATT
1401	ACTGTCGGTA	AAGACGGCAG	TGTAAATCTT	ATTGGTGGCA	AAGTGAAAAA
1451	CGAGGGTGTG	ATTAGCGTAA	ATGGTGGCAG	CATTTCTTTA	CTCGCAGGGC
1501	AAAAAATCAC	CATCAGCGAT	ATAATAAACC	CAACCATTAC	TTACAGCATT
1551	GCCGCGCCTG	AAAATGAAGC	GGTCAATCTG	GGCGATATTT	TTGCCAAAGG

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FIG. 7C.

```
1601 CGGTAACATT AATGTCCGTG CTGCCACTAT TCGAAACCAA GGTAAACTTT
1651 CTGCTGATTC TGTAAGCAAA GATAAAAGCG GCAATATTGT TCTTTCCGCC
1701 AAAGAGGGTG AAGCGGAAAT TGGCGGTGTA ATTTCCGCTC AAAATCAGCA
1751 AGCTAAAGGC GGCAAGCTGA TGATTACAGG CGATAAAGTC ACATTAAAAA
1801 CAGGTGCAGT TATCGACCTT TCAGGTAAAG AAGGGGGAGA AACTTACCTT
1851 GGCGGTGACG AGCGCGGCGA AGGTAAAAAC GGCATTCAAT TAGCAAAGAA
1901 AACCTCTTTA GAAAAAGGCT CAACCATCAA TGTATCAGGC AAAGAAAAAG
1951 GCGGACGCGC TATTGTGTGG GGCGATATTG CGTTAATTGA CGGCAATATT
2001 AACGCTCAAG GTAGTGGTGA TATCGCTAAA ACCGGTGGTT TTGTGGAGAC
2051 ATCGGGGCAT TATTTATCCA TTGACAGCAA TGCAATTGTT AAAACAAAAG
2101 AGTGGTTGCT AGACCCTGAT GATGTAACAA TTGAAGCCGA AGACCCCCTT
2151 CGCAATAATA CCGGTATAAA TGATGAATTC CCAACAGGCA CCGGTGAAGC
2201 AAGCGACCCT AAAAAAAATA GCGAACTCAA AACAACGCTA ACCAATACAA
2251 CTATTTCAAA TTATCTGAAA AACGCCTGGA CAATGAATAT AACGGCATCA
2301 AGAAAACTTA CCGTTAATAG CTCAATCAAC ATCGGAAGCA ACTCCCACTT
2351 AATTCTCCAT AGTAAAGGTC AGCGTGGCGG AGGCGTTCAG ATTGATGGAG
2401 ATATTACTTC TAAAGGCGGA AATTTAACCA TTTATTCTGG CGGATGGGTT
```

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FIG. 7D.

```
2451  GATGTTCATA AAAATATTAC GCTTGATCAG GGTTTTTTAA ATATTACCGC
2501  CGCTTCCGTA GCTTTTGAAG GTGGAAATAA CAAAGCACGC GACGCGGCAA
2551  ATGCTAAAAT TGTCGCCCAG GGCACTGTAA CCATTACAGG AGAGGGAAAA
2601  GATTTCAGGG CTAACAACGT ATCTTTAAAC GGAACGGGTA AAGGTCTGAA
2651  TATCATTTCA TCAGTGAATA ATTTAACCCA CAATCTTAGT GGCACAATTA
2701  ACATATCTGG GAATATAACA ATTAACCAAA CTACGAGAAA GAACACCTCG
2751  TATTGGCAAA CCAGCCATGA TTCGCACTGG AACGTCAGTG CTCTTAATCT
2801  AGAGACAGGC GCAAATTTTA CCTTTATTAA ATACATTTCA AGCAATAGCA
2851  AAGGCTTAAC AACACAGTAT AGAAGCTCTG CAGGGGTGAA TTTTAACGGC
2901  GTAAATGGCA ACATGTCATT CAATCTCAAA GAAGGAGCGA AAGTTAATTT
2951  CAAATTAAAA CCAAACGAGA ACATGAACAC AAGCAAACCT TTACCAATTC
3001  GGTTTTTAGC CAATATCACA GCCACTGGTG GGGGCTCTGT TTTTTTTGAT
3051  ATATATGCCA ACCATTCTGG CAGAGGGGCT GAGTTAAAAA TGAGTGAAAT
3101  TAATATCTCT AACGGCGCTA ATTTTACCTT AAATTCCCAT GTTCGCGGCG
3151  ATGACGCTTT TAAAATCAAC AAAGACTTAA CCATAAATGC AACCAATTCA
3201  AATTTCAGCC TCAGACAGAC GAAAGATGAT TTTTATGACG GGTACGCACG
```

FIG. 7E.

3251	CAATGCCATC	AATTCAACCT	ACAACATATC	CATTCTGGGC	GGTAATGTCA
3301	CCCTTGGTGG	ACAAAACTCA	AGCAGCAGCA	TTACGGGGAA	TATTACTATC
3351	GAGAAAGCAG	CAAATGTTAC	GCTAGAAGCC	AATAACGCCC	CTAATCAGCA
3401	AAACATAAGG	GATAGAGTTA	TAAAACTTGG	CAGCTTGCTC	GTTAATGGGA
3451	GTTTAAGTTT	AACTGGCGAA	AATGCAGATA	TTAAAGGCAA	TCTCACTATT
3501	TCAGAAAGCG	CCACTTTTAA	AGGAAAGACT	AGAGATACCC	TAAATATCAC
3551	CGGCAATTTT	ACCAATAATG	GCACTGCCGA	AATTAATATA	ACACAAGGAG
3601	TGGTAAAACT	TGGCAATGTT	ACCAATGATG	GTGATTTAAA	CATTACCACT
3651	CACGCTAAAC	GCAACCAAAG	AAGCATCATC	GGCGGAGATA	TAATCAACAA
3701	AAAAGGAAGC	TTAAATATTA	CAGACAGTAA	TAATGATGCT	GAAATCCAAA
3751	TTGGCGGCAA	TATCTCGCAA	AAAGAAGGCA	ACCTCACGAT	TTCTTCCGAT
3801	AAAATTAATA	TCACCAAACA	GATAACAATC	AAAAAGGGTA	TTGATGGAGA
3851	GGACTCTAGT	TCAGATGCGA	CAAGTAATGC	CAACCTAACT	ATTAAAACCA
3901	AAGAATTGAA	ATTGACAGAA	GACCTAAGTA	TTTCAGGTTT	CAATAAAGCA
3951	GAGATTACAG	CCAAAGATGG	TAGAGATTTA	ACTATTGGCA	ACAGTAATGA
4001	CGGTAACAGC	GGTGCCGAAG	CCAAAACAGT	AACTTTTAAC	AATGTTAAAG

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FIG.7F.

4051 ATTCAAAAAT CTCTGCTGAC GGTCACAATG TGACACTAAA TAGCAAAGTG
4101 AAAACATCTA GCAGCAATGG CGGACGTGAA AGCAATAGCG ACAACGATAC
4151 CGGCTTAACT ATTACTGCAA AAAATGTAGA AGTAAACAAA GATATTACTT
4201 CTCTCAAAAC AGTAAATATC ACCGCGTCGG AAAAGGTTAC CACCACAGCA
4251 GGCTCGACCA TTAACGCAAC AAATGGCAAA GCAAGTATTA CAACCAAAAC
4301 AGGTGATATC AGCGGTACGA TTTCCGGTAA CACGGTAAGT GTTAGCGCGA
4351 CTGGTGATTT AACCACTAAA TCCGGCTCAA AAATTGAAGC GAAATCGGGT
4401 GAGGCTAATG TAACAAGTGC AACAGGTACA ATTGGCGGTA CAATTTCCGG
4451 TAATACGGTA AATGTTACGG CAAACGCTGG CGATTTAACA GTTGGGAATG
4501 GCGCAGAAAT TAATGCGACA GAAGGAGCTG CAACCTTAAC CGCAACAGGG
4551 AATACCTTGA CTACTGAAGC CGGTTCTAGC ATCACTTCAA CTAAGGGTCA
4601 GGTAGACCTC TTGGCTCAGA ATGGTAGCAT CGCAGGAAGC ATTAATGCTG
4651 CTAATGTGAC ATTAAATACT ACAGGCACCT TAACCACCGT GGCAGGCTCG
4701 GATATTAAAG CAACCAGCGG CACCTTGGTT ATTAACGCAA AAGATGCTAA
4751 GCTAAATGGT GATGCATCAG GTGATAGTAC AGAAGTGAAT GCAGTCAACG
4801 ACTGGGGATT TGGTAGTGTG ACTGCGGCAA CCTCAAGCAG TGTGAATATC
4851 ACTGGGGATT TAAACACAGT AAATGGGTTA AATATCATTT CGAAAGATGG

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FIG. 7G.

```
4901  TAGAAACACT GTGCGCTTAA GAGGCAAGGA AATTGAGGTG AAATATATCC
4951  AGCCAGGTGT AGCAAGTGTA GAAGAAGTAA TTGAAGCGAA ACGCGTCCTT
5001  GAAAAAGTAA AAGATTTATC TGATGAAGAA AGAGAAACAT TAGCTAAACT
5051  TGGTGTAAGT GCTGTACGTT TTGTTGAGCC AAATAATACA ATTACAGTCA
5101  ATACACAAAA TGAATTTACA ACCAGACCGT CAAGTCAAGT GATAATTTCT
5151  GAAGGTAAGG CGTGTTTCTC AAGTGGTAAT GGCGCACGAG TATGTACCAA
5201  TGTTGCTGAC GATGGACAGC CGTAGTCAGT AATTGACAAG GTAGATTTCA
5251  TCCTGCAATG AAGTCATTTT ATTTTCGTAT TATTTACTGT GTGGGTTAAA
5301  GTTCAGTACG GGCTTTACCC ATCTTGTAAA AAATTACGGA GAATACAATA
5351  AAGTATTTTT AACAGGTTAT TATTATGAAA AATATAAAAA GCAGATTAAA
5401  ACTCAGTGCA ATATCAGTAT TGCTTGGCCT GGCTTCTTCA TCATTGTATG
5451  CAGAAGAAGC GTTTTTAGTA AAAGGCTTTC AGTTATCTGG TGCACTTGAA
5501  ACTTTAAGTG AAGACGCCCA ACTGTCTGTA GCAAAATCTT TATCTAAATA
5551  CCAAGGCTCG CAAACTTTAA CAAACCTAAA AACAGCACAG CTTGAATTAC
5601  AGGCTGTGCT AGATAAGATT GAGCCAAATA AATTTGATGT GATATTGCCG
5651  CAACAAACCA TTACGGATGG CAATATCATG TTTGAGCTAG TCTCGAAATC
```

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FIG. 7H.

```
5701  AGCCGCAGAA AGCCAAGTTT TTTATAAGGC GAGCCAGGGT TATAGTGAAG
5751  AAAATATCGC TCGTAGCCTG CCATCTTTGA AACAAGGAAA AGTGTATGAA
5801  GATGGTCGTC AGTGGTTCGA TTTGCGTGAA TTTAATATGG CAAAAGAAAA
5851  CCCGCTTAAG GTTACCCGTG TACATTACGA ACTAAACCCT AAAAACAAAA
5901  CCTCTAATTT GATAATTGCG GGCTTCTCGC CTTTTGGTAA AACGCGTAGC
5951  TTTATTTCTT ATGATAATTT CGGCGCGAGA GAGTTTAACT ACCAACGTGT
6001  AAGCTTGGGT TTTGTTAATG CCAATTTAAC TGGTCATGAT GATGTGTTAA
6151  TTATACCAGT ATGAGTTATG CTGATTCTAA TGATATCGAC GGCTTACCAA
6201  GTGCGATTAA TCGTAAATTA TCAAAAGGTC AATCTATCTC TGCGAATCTG
6251  AAATGGAGTT ATTATCTCCC AACATTTAAC CTTGGCATGG AAGACCAATT
6301  TAAAATTAAT TTAGGCTACA ACTACCGCCA TATTAATCAA ACCTCCGCGT
6351  TAAATCGCTT GGGTGAAACG AAGAAAAAAT TTGCAGTATC AGGCGTAAGT
6401  GCAGGCATTG ATGGACATAT CCAATTTACC CCTAAAACAA TCTTTAATAT
6451  TGATTTAACT CATCATTATT ACGCGAGTAA ATTACCAGGC TCTTTTGGAA
6501  TGGAGCGCAT TGGCGAAACA TTTAATCGCA GCTATCACAT TAGCACAGCC
6551  AGTTTAGGGT TGAGTCAAGA GTTTGCTCAA GGTTGGCATT TTAGCAGTCA
6601  ATTATCAGGT CAATTTACTC TACAAGATAT TAGCAGTATA GATTTATTCT
```

FIG. 7I.

6651	CTGTAACAGG	TACTTATGGC	GTCAGAGGCT	TTAAATACGG	CGGTGCAAGT
6701	GGTGAGCGCG	GTCTTGTATG	GCGTAATGAA	TTAAGTATGC	CAAAATACAC
6751	CCGCTTCCAA	ATCAGCCCTT	ATGCGTTTTA	TGATGCAGGT	CAGTTCCGTT
6801	ATAATAGCGA	AAATGCTAAA	ACTTACGGCG	AAGATATGCA	CACGGTATCC
6851	TCTGCGGGTT	TAGGCATTAA	AACCTCTCCT	ACACAAAACT	TAAGCCTAGA
6901	TGCTTTTGTT	GCTCGTCGCT	TTGCAAATGC	CAATAGTGAC	AATTTGAATG
6951	GCAACAAAAA	ACGCACAAGC	TCACCTACAA	CCTTCTGGGG	GAGATTAACA
7001	TTCAGTTTCT	AACCCTGAAA	TTTAATCAAC	TGGTAAGCGT	TCCGCCTACC
7051	AGTTTATAAC	TATATGCTTT	ACCCGCCAAT	TTACAGTCTA	TAGGCAACCC
7101	TGTTTTTACC	CTTATATATC	AAATAAACAA	GCTAAGCTGA	GCTAAGCAAA
7151	CCAAGCAAAC	TCAAGCAAGC	CAAGTAATAC	TAAAAAAACA	ATTTATATGA
7201	TAAACTAAAG	TATACTCCAT	GCCATGGCGA	TACAAGGGAT	TTAATAATAT
7251	GACAAAAGAA	AATTTGCAAA	ACGCTCCTCA	AGATGCGACC	GCTTTACTTG
7301	CGGAATTAAG	CAACAATCAA	ACTCCCCTGC	GAATATTTAA	ACAACCACGC
7351	AAGCCCAGCC	TATTACGCTT	GGAACAACAT	ATCGCAAAAA	AAGATTATGA
7401	GTTTGCTTGT	CGTGAATTAA	TGGTGATTCT	GGAAAAAATG	GACGCTAATT

FIG. 7J.

```
7451  TTGGAGGCGT TCACGATATT GAATTTGACG CACCCGCTCA GCTGGCATAT
7501  CTACCCGAAA AATTACTAAT TTATTTTGCC ACTCGTCTCG CTAATGCAAT
7551  TACAACACTC TTTTCCGACC CCGAATTGGC AATTTCTGAA GAAGGGGCGT
7601  TAAAGATGAT TAGCCTGCAA CGCTGGTTGA CGCTGATTTT TGCCTCTTCC
7651  CCCTACGTTA ACGCAGACCA TATTCTCAAT AAATATAATA TCAACCCAGA
7701  TTCCGAAGGT GGCTTTCATT TAGCAACAGA CAACTCTTCT ATTGCTAAAT
7751  TCTGTATTTT TTACTTACCC GAATCCAATG TCAATATGAG TTTAGATGCG
7801  TTATGGGCAG GGAATCAACA ACTTTGTGCT TCATTGTGTT TTGCGTTGCA
7851  GTCTTCACGT TTTATTGGTA CCGCATCTGC GTTTCATAAA AGAGCGGTGG
7901  TTTTACAGTG GTTTCCTAAA AAACTCGCCG AAATTGCTAA TTTAGATGAA
7951  TTGCCTGCAA ATATCCTTCA TGATGTATAT ATGCACTGCA GTTATGATTT
8001  AGCAAAAAAC AAGCACGATG TTAAGCGTCC ATTAAACGAA CTTGTCCGCA
8051  AGCATATCCT CACGCAAGGA TGGCAAGACC GCTACCTTTA CACCTTAGGT
8101  AAAAAGGACG GCAAACCTGT GATGATGGTA CTGCTTGAAC ATTTTAATTC
8151  GGGACATTCG ATTTATCGTA CACATTCAAC TTCAATGATT GCTGCTCGAG
8201  AAAAATTCTA TTTAGTCGGC TTAGGCCATG AGGGCGTTGA TAAAATAGGT
```

FIG.7K.

```
8251  CGAGAAGTGT TTGACGAGTT CTTTGAAATC AGTAGCAATA ATATAATGGA
8301  GAGACTGTTT TTTATCCGTA AACAGTGCGA AACTTTCCAA CCCGCAGTGT
8351  TCTATATGCC AAGCATTGGC ATGGATATTA CCACGATTTT TGTGAGCAAC
8401  ACTCGGCTTG CCCCTATTCA AGCTGTAGCC CTGGGTCATC CTGCCACTAC
8451  GCATTCTGAA TTTATTGATT ATGTCATCGT AGAAGATGAT TATGTGGGCA
8501  GTGAAGATTG TTTCAGCGAA ACCCTTTTAC GCTTACCCAA AGATGCCCTA
8551  CCTTATGTAC CTTCTGCACT CGCCCCACAA AAAGTGGATT ATGTACTCAG
8601  GGAAAACCCT GAAGTAGTCA ATATCGGTAT TGCCGCTACC ACAATGAAAT
8651  TAAACCCTGA ATTTTTGCTA ACATTGCAAG AAATCAGAGA TAAAGCTAAA
8701  GTCAAAATAC ATTTTCATTT CGCACTTGGA CAATCAACAG GCTTGACACA
8751  CCCTTATGTC AAATGGTTTA TCGAAAGCTA TTTAGGTGAC GATGCCACTG
8801  CACATCCCCA CGCACCTTAT CACGATTATC TGGCAATATT GCGTGATTGC
8851  GATATGCTAC TAAATCCGTT TCCTTTCGGT AATACTAACG GCATAATTGA
8901  TATGGTTACA TTAGGTTTAG TTGGTGTATG CAAAACGGGG GATGAAGTAC
8951  ATGAACATAT TGATGAAGGT CTGTTTAAAC GCTTAGGACT ACCAGAATGG
9001  CTGATAGCCG ACACACGAGA AACATATATT GAATGTGCTT TGCGTCTAGC
9051  AGAAAACCAT CAAGAACGCC TTGAACTCCG TCGTTACATC ATAGAAAACA
```

FIG. 7L.

```
9101  ACGGCTTACA AAAGCTTTTT ACAGGCGACC CTCGTCCATT GGGCAAAATA
9151  CTGCTTAAGA AAACAAATGA ATGGAAGCGG AAGCACTTGA GTAAAAAATA
9201  ACGGTTTTTT AAAGTAAAAG TGCGGTTAAT TTTCAAAGCG TTTTAAAAAC
9251  CTCTCAAAAA TCAACCGCAC TTTTATCTTT ATAACGATCC CGCACGCTGA
9301  CAGTTTATCA GCCTCCCGCC ATAAAACTCC GCCTTTCATG GCGGAGATTT
9351  TAGCCAAAAC TGGCAGAAAT TAAAGGCTAA AATCACCAAA TTGCACCACA
9401  AAATCACCAA TACCCACAAA AAA
```

HMW3 nucleotide sequence

Fig. 8 Fig 8A

REFORMAT of: Temp3.Gcg check: -1 from: 1 to: 4794 October 5, 1995 17:43

(No documentation)

Hmw3.Gcg Length: 4794 October 5, 1995 18:29 Type: N Check: 484 ..

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   1 ATGAACAAGA TATATCGTCT CAAATTCAGC AAACGCCTGA ATGCTTTGGT TGCTGTGTCT GAATTGACAC GGGGTTGTGA CCATTCCACA GAAAAAGGCA
 101 GTGAAAAACC TGTTCGTACG AAAGTACGCC ACTTGGCGTT AAAGCCACTT TCCGCTATAT TGCTATCTTT GGGCATGGCA TCCATTCCGC AATCTGTTTT
 201 AGCGAGCGGT TTACAGGGAA TGAGCGTCGT ACACGGTACA GCAACCATGC AAGTAGACGG CAATAAAACC ACTATCCGTA ATAGCGTCAA TGCTATCATC
 301 AATTGGAAAC AATTTAACAT TGACCAAAAT GAAATGGTGC AGTTTTTACA AGAAAGCAGC AACTCTGCCG TTTTCAACCG TGTTACATCT GACCAAATCT
 401 CCCAATTAAA AGGGATTTTA GATTCTAACG GACAAGTCTT TTTAATCAAC CCAAATGGTA TCACAATAGG TAAAGACGCA ATTATTAACA CTAATGGCTT
 501 TACTGCTTCT ACGCTAGACA TTTCTAACGA AAACATCAAG GCGCGTAATT TCACCCTTGA GCAAACCAAG GATAAAGCAC TCGCTGAAAT CGTGAATCAC
 601 GGTTTAATTA CCGTTGGTAA AGACGGTAGC GTAAACCTTA TTGGTGGCAA AGTGAAAAAC GAGGGCGTGA TTAGCGTAAA TGGCGGTAGT ATTTCTTTAC
 701 TTGCAGGGCA AAAAATCACC ATCAGCGATA TAATAAATCC AACCATCACT TACAGCATTG CTGCACCTGA AAACGAAGCG ATCAATCTGG GCGATATTTT
 801 TGCCAAAGGT GGTAACATTA ATGTCCGCGC TGCCACTATT CGCAATAAAG GTAAACTTTC TGCCGACTCT GTAAGCAAAG ATAAAAGTGG TAACATTGTT
 901 CTCTCTGCCA AAGAAGGTGA AGCGGAAATT GGCGGTGTAA TTTCCGCTCA AAATCAGCAA GCCAAAGGTG GTAAGTTGAT GATTACAGGC GATAAAGTTA
1001 CATTGAAAAC GGGTGCAGTT ATCGACCTTT CGGGTAAAGA AGGGGGAGAA ACTTATCTTG GCGGTGACGA GCGTGGCGAA GGTAAAAACG GCATTCAATT
1101 AGCAAAGAAA ACCACTTTAG AAAAAGGCTC AACAATTAAT GTGTCAGGTA AAGAAAAAGG TGGGCGCGCT ATTGTATGGG GCGATATTGC GTTAATTGAC
1201 GGCAATATTA ATGCCCAAGG TAAAGATATC GCTAAAACTG GTGGTTTTGT GGAGACGTCG GGGCATTACT TATCCATTGA TGATAACGCA ATTGTTAAAA
1301 CAAAAGAATG GCTACTAGAC CCAGAGAATG TGACTATTGA AGCTCCTTCC GCTTCTCGCG TCGAGCTGGG TGCCGATAGG AATTCCCACT CGGCAGAGGT
1401 GATAAAAGTG ACCCTAAAAA AAAATAACAC CTCCTTGACA ACACTAACCA ATACAACCAT TTCAAATCTT CTGAAAAGTG CCCACGTGGT GAACATAACG
1501 GCAAGGAGAA AACTTACCGT TAATAGCTCT ATCAGTATAG AAAGAGGCTC CCACTTAATT CTCCACAGTG AAGGTCAGGG CGGTCAAGGT GTTCAGATTG
1601 ATAAAGATAT TACTTCTGAA GGCGGAAATT-TAACCATTTA TTCTGGCGGA TGGGTTGATG TTCATAAAAA TATTACGCTT GGTAGCGGCT TTTTAAACAT
1701 CACAACTAAA GAAGGAGATA TCGCCTTCGA AGACAAGTCT GGACGGAACA ACCTAACCAT TACAGCCCAA GGGACCATCA CCTCAGGTAA TAGTAACGGC
1801 TTTAGATTTA ACAACGTCTC TCTAAACAGC CTTGGCGGAA AGCTGAGCTT TACTGACAGC AGAGAGGACA GAGGTAGAAG AACTAAGGGT AATATCTCAA
1901 ACAAATTTGA CGGAACGTTA AACATTTCCG GAACTGTAGA TATCTCAATG AAAGCACCCA AAGTCAGCTG GTTTTACAGA GACAAAGGAC GCACCTACTG
2001 GAACGTAACC ACTTTAAATG TTACCTCGGG TAGTAAATTT AACCTCTCCA TTGACAGCAC AGGAAGTGGC TCAACAGGTC CAAGCATACG CAATGCAGAA
2101 TTAAATGGCA TAACATTTAA TAAAGCCACT TTTAATATCG CACAAGGCTC AACAGCTAAC TTTAGCATCA AGGCATCAAT AATGCCCTTT AAGAGTAACG
2201 CTAACTACGC ATTATTTAAT GAAGATATTT CAGTCTCAGG GGGGGGTAGC GTTAATTTCA AACTTAACGC CTCATCTAGC AACATACAAA CCCCTGGCGT
2301 AATTATAAAA TCTCAAAACT TTAATGTCTC AGGAGGGTCA ACTTTAAATC TCAAGGCTGA AGGTTCAACA GAAACCGCTT TTTCAATAGA AAATGATTTA
2401 AACTTAAACG CCACCGGTGG CAATATAACA ATCAGACAAG TCGAGGGTAC CGATTCACGC GTCAACAAAG GTGTCGCAGC CAAAAAAAAC ATAACTTTTA
2501 AAGGGGGTAA TATCACCTTC GGCTCTCAAA AAGCCACAAC AGAAATCAAA GGCAATGTTA CCATCAATAA AAACACTAAC GCTACTCTTC GTGGTGCGAA
```

2601 TTTTGCCGAA AACAAATCGC CTTTAAATAT AGCAGGAAAT GTTATTAATA ATGGCAACCT TACCACTGCC GGCTCCATTA TCAATATAGC CGGAAATCTT

2701 ACTGTTTCAA AAGGCGCTAA CCTTCAAGCT ATAACAAATT ACACTTTTAA TGTAGCCGGC TCATTTGACA ACAATGGCGC TTCAAACATT TCCATTGCCA

2801 GAGGAGGGGC TAAATTTAAA GATATCAATA ACACCAGTAG CTTAAATATT ACCACCAACT CTGATACCAC TTACCGCACC ATTATAAAAG GCAATATATC

2901 CAACAAATCA GGTGATTTGA ATATTATTGA TAAAAAAAGC GACGCTGAAA TCCAAATTGG CGGCAATATC TCACAAAAAG AAGGCAATCT CACAATTTCT

3001 TCTGATAAAG TAAATATTAC CAATCAGATA ACAATCAAAG CAGGCGTTGA AGGGGGGCGT TCTGATTCAA GTGAGGCAGA AAATGCTAAC CTAACTATTC

3101 AAACCAAAGA GTTAAAATTG GCAGGAGACC TAAATATTTC AGGCTTTAAT AAAGCAGAAA TTACAGCTAA AAATGGCAGT GATTTAACTA TTGGCAATGC

3201 TAGCGGTGGT AATGCTGATG CTAAAAAAGT GACTTTTGAC AAGGTTAAAG ATTCAAAAAT CTCGACTGAC GGTCACAATG TAACACTAAA TAGCGAAGTG

3301 AAAACGTCTA ATGGTAGTAG CAATGCTGGT AATGATAACA GCACCGGTTT AACCATTTCC GCAAAAGATG TAACGGTAAA CAATAACGTT ACCTCCCACA

3401 AGACAATAAA TATCTCTGCC GCAGCAGGAA ATGTAACAAC CAAAGAAGGC ACAACTATCA ATGCAACCAC AGGCAGCGTG GAAGTAACTG CTCAAAATGG

3501 TACAATTAAA GGCAACATTA CCTCGCAAAA TGTAACAGTG ACAGCAACAG AAAATCTTGT TACCACAGAG AATGCTGTCA TTAATGCAAC CAGCGGCACA

3601 GTAAACATTA GTACAAAAAC AGGGGATATT AAAGGTGGAA TTGAATCAAC TTCCGGTAAT GTAAATATTA CAGCGAGCGG CAATACACTT AAGGTAAGTA

3701 ATATCACTGG TCAAGATGTA ACAGTAACAG CGGATGCAGG AGCCTTGACA ACTACAGCAG GCTCAACCAT TAGTGCGACA ACAGGCAATG CAAATATTAC

3801 AACCAAAACA GGTGATATCA ACGGTAAAGT TGAATCCAGC TCCGGCTCTG TAACACTTGT TGCAACTGGA GCAACTCTTG CTGTAGGTAA TATTTCAGGT

3901 AACACTGTTA CTATTACTGC GGATAGCGGT AAATTAACCT CCACAGTAGG TTCTACAATT AATGGGACTA ATAGTGTAAC CACCTCAAGC CAATCAGGCG

4001 ATATTGAAGG TACAATTTCT GGTAATACAG TAAATGTTAC AGCAAGCACT GGTGATTTAA CTATTGGAAA TAGTGCAAAA GTTGAAGCGA AAAATGGAGC

4101 TGCAACCTTA ACTGCTGAAT CAGGCAAATT AACCACCCAA ACAGGCTCTA GCATTACCTC AAGCAATGGT CAGACAACTC TTACAGCCAA GGATAGCAGT

4201 ATCGCAGGAA ACATTAATGC TGCTAATGTG ACGTTAAATA CCACAGGCAC TTTAACTACT ACAGGGGATT CAAAGATTAA CGCAACCAGT GGTACCTTAA

4301 CAATCAATGC AAAAGATGCC AAATTAGATG GTGCTGCATC AGGTGACCGC ACAGTAGTAA ATGCAACTAA CGCAAGTGGC TCTGGTAACG TGACTGCGAA

4401 AACCTCAAGC AGCGTGAATA TCACCGGGGA TTTAAACACA ATAAATGGGT TAAATATCAT TTCGGAAAAT GGTAGAAACA CTGTGCGCTT AAGAGGCAAG

4501 GAAATTGATG TGAAATATAT CCAACCAGGT GTAGCAAGCG TAGAAGAGGT AATTGAAGCG AAACGCGTCC TTGAGAAGGT AAAAGATTTA TCTGATGAAG

4601 AAAGAGAAAC ACTAGCCAAA CTTGGTGTAA GTGCTGTACG TTTCGTTGAG CCAAATAATG CCATTACGGT TAATACACAA AACGAGTTTA CAACCAAACC

4701 ATCAAGTCAA GTGACAATTT CTGAAGGTAA GGCGTGTTTC TCAAGTGGTA ATGGCGCACG AGTATGTACC AATGTTGCTG ACGATGGACA GCAG

Fig 9

HMW4 nucleotide sequence

Fig 9A

REFORMAT of: Temp4.Gcg check: -1 from: 1 to: 4803 October 5, 1995 17:44

(No documentation)

Hmw4.Gcg Length: 4803 October 5, 1995 18:29 Type: N Check: 3920 ..

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   1 ATGAACAAGA TATATCGTCT CAAATTCAGC AAACGCCTGA ATGCTTTGGT TGCTGTGTCT GAATTGACAC GGGGTTGTGA CCATTCCACA GAAAAAGGCA
 101 GTGAAAAACC TGTTCGTACG AAAGTACGCC ACTTGGCGTT AAAGCCACTT TCCGCTATAT TGCTATCTTT GGGCATGGCA TCCATTCCGC AATCTGTTTT
 201 AGCGAGCGGT TTACAGGGAA TGAGCGTCGT ACACGGTACA GCAACCATGC AAGTAGACGG CAATAAAACC ACTATCCGTA ATAGCGTCAA TGCTATCATC
 301 AATTGGAAAC AATTTAACAT TGACCAAAAT GAAATGGTGC AGTTTTTACA AGAAAGCAGC AACTCTGCCG TTTTCAACCG TGTTACATCT GACCAAATCT
 401 CCCAATTAAA AGGGATTTTA GATTCTAACG GACAAGTCTT TTTAATCAAC CCAAATGGTA TCACAATAGG TAAAGACGCA ATTATTAACA CTAATGGCTT
 501 TACTGCTTCT ACGCTAGACA TTTCTAACGA AAACATCAAG GCGCGTAATT TCACCCTTGA GCAAACCAAG GATAAAGCAC TCGCTGAAAT CGTGAATCAC
 601 GGTTTAATTA CCGTTGGTAA AGACGGTAGC GTAAACCTTA TTGGTGGCAA AGTGAAAAAC GAGGGCGTGA TTAGCGTAAA TGGCGGTAGT ATTTCTTTAC
 701 TTGCAGGGCA AAAAATCACC ATCAGCGATA TAATAAATCC AACCATCACT TACAGCATTG CTGCACCTGA AAACGAAGCG ATCAATCTGG GCGATATTTT
 801 TGCCAAAGGT GGTAACATTA ATGTCCGCGC TGCCACTATT CGCAATAAAG GTAAACTTTC TGCCGACTCT GTAAGCAAAG ATAAAAGTGG TAACATTGTT
 901 CTCTCTGCCA AAGAAGGTGA AGCGGAAATT GGCGGTGTAA TTTCCGCTCA AAATCAGCAA GCCAAAGGTG GTAAGTTGAT GATTACAGGT GATAAAGTCA
1001 CATTAAAAAC AGGTGCAGTT ATCGACCTTT CAGGTAAAGA AGGGGGAGAG ACTTATCTTG GCGGTGATGA GCGTGGCGAA GGTAAAAATG GTATTCAATT
1101 AGCGAAGAAA ACCTCTTTAG AAAAAGGCTC GACAATTAAT GTATCAGGCA AAGAAAAAGG CGGGCGCGCT ATTGTATGGG GCGATATTGC ATTAATTAAT
1201 GGTAACATTA ATGCTCAAGG TAGCGATATT GCTAAAACTG GCGGCTTTGT GGAAACATCA GGACATGACT TATCCATTGG TGATGATGTG ATTGTTGACG
1301 CTAAAGAGTG GTTATTAGAC CCAGATGATG TGTCCATTGA AACTCTTACA TCTGGACGCA ATAATACCGG CGAAAACCAA GGATATACAA CAGGAGATGG
1401 GACTAAAGAG TCACCTAAAG GTAATAGTAT TTCTAAACCT ACATTAACAA ACTCAACTCT TGAGCAAATC CTAAGAAGAG GTTCTTATGT TAATATCACT
1501 GCTAATAATA GAATTTATGT TAATAGCTCC ATCAACTTAT CTAATGGCAG TTTAACACTT CACACTAAAC GAGATGGAGT TAAAATTAAC GGTGATATTA
1601 CCTCAAACGA AAATGGTAAT TTAACCATTA AAGCAGGCTC TTGGGTTGAT GTTCATAAAA ACATCACGCT TGGTACGGGT TTTTTGAATA TTGTCGCTGG
1701 GGATTCTGTA GCTTTTGAGA GAGAGGGCGA TAAAGCACGT AACGCAACAG ATGCTCAAAT TACCGCACAA GGGACGATAA CCGTCAATAA AGATGATAAA
1801 CAATTTAGAT TCAATAATGT ATCTATTAAC GGGACGGGCA AGGGTTTAAA GTTTATTGCA AATCAAAATA ATTTCACTCA TAAATTTGAT GGCGAAATTA
1901 ACATATCTGG AATAGTAACA ATTAACCAAA CCACGAAAAA AGATGTTAAA TACTGGAATG CATCAAAAGA CTCTTACTGG AATGTTTCTT CTCTTACTTT
2001 GAATACGGTG CAAAAATTTA CCTTTATAAA ATTCGTTGAT AGCGGCTCAA ATTCCCAAGA TTTGAGGTCA TCACGTAGAA GTTTTGCAGG CGTACATTTT
2101 AACGGCATCG GAGGCAAAAC AAACTTCAAC ATCGGAGCTA ACGCAAAAGC CTTATTTAAA TTAAAACCAA ACGCCGCTAC AGACCCAAAA AAAGAATTAC
2201 CTATTACTTT TAACGCCAAC ATTACAGCTA CCGGTAACAG TGATAGCTCT GTGATGTTTG ACATACACGC CAATCTTACC TCTAGAGCTG CCGGCATAAA
2301 CATGGATTCA ATTAACATTA CCGGCGGGCT TGACTTTTCC ATAACATCCC ATAATCGCAA TAGTAATGCT TTTGAAATCA AAAAAGACTT AACTATAAAT
2401 GCAACTGGCT CGAATTTTAG TCTTAAGCAA ACGAAAGATT CTTTTTATAA TGAATACAGC AAACACGCCA TTAACTCAAG TCATAATCTA ACCATTCTTG
```

Fig 4B

2501 GCGGCAATGT CACTCTAGGT GGGGAAAATT CAAGCAGTAG CATTACGGGC AATATCAATA TCACCAATAA AGCAAATGTT ACATTACAAG CTGACACCAG
2601 CAACAGCAAC ACAGGCTTGA AGAAAAGAAC TCTAACTCTT GGCAATATAT CTGTTGAGGG GAATTTAAGC CTAACTGGTG CAAATGCAAA CATTGTCGGC
2701 AATCTTTCTA TTGCAGAAGA TTCCACATTT AAAGGAGAAG CCAGTGACAA CCTAAACATC ACCGGCACCT TTACCAACAA CGGTACCGCC AACATTAATA
2801 TAAAACAAGG AGTGGTAAAA CTCCAAGGCG ATATTATCAA TAAAGGTGGT TTAAATATCA CTACTAACGC CTCAGGCACT CAAAAAACCA TTATTAACGG
2901 AAATATAACT AACGAAAAAG GCGACTTAAA CATCAAGAAT ATTAAAGCCG ACGCCGAAAT CCAAATTGGC GGCAATATCT CACAAAAAGA AGGCAATCTC
3001 ACAATTTCTT CTGATAAAGT AAATATTACC AATCAGATAA CAATCAAAGC AGGCGTTGAA GGGGGGCGTT CTGATTCAAG TGAGGCAGAA AATGCTAACC
3101 TAACTATTCA AACCAAAGAG TTAAAATTGG CAGGAGACCT AAATATTTCA GGCTTTAATA AAGCAGAAAT TACAGCTAAA AATGGCAGTG ATTTAACTAT
3201 TGGCAATGCT AGCGGTGGTA ATGCTGATGC TAAAAAAGTG ACTTTTGACA AGGTTAAAGA TTCAAAAATC TCGACTGACG GTCACAATGT AACACTAAAT
3301 AGCGAAGTGA AAACGTCTAA TGGTAGTAGC AATGCTGGTA ATGATAACAG CACCGGTTTA ACCATTTCCG CAAAAGATGT AACGGTAAAC AATAACGTTA
3401 CCTCCCACAA GACAATAAAT ATCTCTGCCG CAGCAGGAAA TGTAACAACC AAAGAAGGCA CAACTATCAA TGCAACCACA GGCAGCGTGG AAGTAACTGC
3501 TCAAAATGGT ACAATTAAAG GCAACATTAC CTCGCAAAAT GTAACAGTGA CAGCAACAGA AAATCTTGTT ACCACAGAGA ATGCTGTCAT TAATGCAACC
3601 AGCGGCACAG TAAACATTAG TACAAAAACA GGGGATATTA AAGGTGGAAT TGAATCAACT TCCGGTAATG TAAATATTAC AGCGAGCGGC AATACACTTA
3701 AGGTAAGTAA TATCACTGGT CAAGATGTAA CAGTAACAGC GGATGCAGGA GCCTTGACAA CTACAGCAGG CTCAACCATT AGTGCGACAA CAGGCAATGC
3801 AAATATTACA ACCAAAACAG GTGATATCAA CGGTAAAGTT GAATCCAGCT CCGGCTCTGT AACACTTGTT GCAACTGGAG CAACTCTTGC TGTAGGTAAT
3901 ATTTCAGGTA ACACTGTTAC TATTACTGCG GATAGCGGTA AATTAACCTC CACAGTAGGT TCTACAATTA ATGGGACTAA TAGTGTAACC ACCTCAAGCC
4001 AATCAGGCGA TATTGAAGGT ACAATTTCTG GTAATACAGT AAATGTTACA GCAAGCACTG GTGATTTAAC TATTGGAAAT AGTGCAAAAG TTGAAGCGAA
4101 AAATGGAGCT GCAACCTTAA CTGCTGAATC AGGCAAATTA ACCACCCAAA CAGGCTCTAG CATTACCTCA AGCAATGGTC AGACAACTCT TACAGCCAAG
4201 GATAGCAGTA TCGCAGGAAA CATTAATGCT GCTAATGTGA CGTTAAATAC CACAGGCACT TTAACTACTA CAGGGGATTC AAAGATTAAC GCAACCAGTG
4301 GTACCTTAAC AATCAATGCA AAAGATGCCA AATTAGATGG TGCTGCATCA GGTGACCGCA CAGTAGTAAA TGCAACTAAC GCAAGTGGCT CTGGTAACGT
4401 GACTGCGAAA ACCTCAAGCA GCGTGAATAT CACCGGGGAT TTAAACACAA TAAATGGGTT AAATATCATT TCGGAAAATG GTAGAAACAC TGTGCGCTTA
4501 AGAGGCAAGG AAATTGATGT GAAATATATC CAACCAGGTG TAGCAAGCGT AGAAGAGGTA ATTGAAGCGA AACGCGTCCT TGAGAAGGTA AAAGATTTAT
4601 CTGATGAAGA AAGAGAAACA CTAGCCAAAC TTGGTGTAAG TGCTGTACGT TTCGTTGAGC CAAATAATGC CATTACGGTT AATACACAAA ACGAGTTTAC
4701 AACCAAACCA TCAAGTCAAG TGACAATTTC TGAAGGTAAG GCGTGTTTCT CAAGTGGTAA TGGCGCACGA GTATGTACCA ATGTTGCTGA CGATGGACAG
4801 CAG

FIG.10A. COMPARISON OF DERIVED AMINO ACID SEQUENCE

	1				50
Hmw3com	MNKIYRLKFS	KRLNALVAVS	ELTRGCDHST	EKGSEKPYRT	KVRHLALKPL
Hmw4com	MNKIYRLKFS	KRLNALVAVS	ELTRGCDHST	EKGSEKPYRT	KVRHLALKPL
Hmw1com	MNKIYRLKFS	KRLNALVAVS	ELARGCDHST	EKGSEKPARM	KVRHLALKPL
Hmw2com	MNKIYRLKFS	KRLNALVAVS	ELARGCDHST	EKGSEKPARM	KVRHLALKPL

	51				100
Hmw3com	SAILLSLGMA	SIPQSVLASG	LQGMSVVHGT	ATMQVDGNKT	TIRNSVNAII
Hmw4com	SAILLSLGMA	SIPQSVLASG	LQGMSVVHGT	ATMQVDGNKT	TIRNSVNAII
Hmw1com	SAMLLSLGVT	SIPQSVLASG	LQGMSVVHGT	ATMQVDGNKT	TIRNSVNAII
Hmw2com	SAMLLSLGVT	SIPQSVLASG	LQGMSVVHGT	ATMQVDGNKT	TIRNSVNAII

	101				150
Hmw3com	NWKQFNIDQN	EMEQFLQESS	NSAVFNRVTS	DQISQLKGIL	DSNGQVFLIN
Hmw4com	NWKQFNIDQN	EMEQFLQESS	NSAVFNRVTS	DQISQLKGIL	DSNGQVFLIN

FIG.10B.

```
Hmw1com    NWKQFNIDQN EMVQFLQENN NSAVFNRVTS NQISQLKGIL DSNGQVFLIN
Hmw2com    NWKQFNIDQN EMVQFLQENN NSAVFNRVTS NQISQLKGIL DSNGQVFLIN

           151                                                 200
Hmw3com    PNGITIGKDA IINTNGFTAS TLDISNENIK ARNFTLEQTK DKALAEIVNH
Hmw4com    PNGITIGKDA IINTNGFTAS TLDISNENIK ARNFTLEQTK DKALAEIVNH
Hmw1com    PNGITIGKDA IINTNGFTAS TLDISNENIK ARNFTLEQTK DKALAEIVNH
Hmw2com    PNGITIGKDA IINTNGFTAS TLDISNENIK ARNFTLEQTK DKALAEIVNH

           201                                                 250
Hmw3com    GLITVGKDGS VNLIGGKVKN EGVISVNGGS ISLLAGQKIT ISDIINPTIT
Hmw4com    GLITVGKDGS VNLIGGKVKN EGVISVNGGS ISLLAGQKIT ISDIINPTIT
Hmw1com    GLITVGKDGS VNLIGGKVKN EGVISVNGGS ISLLAGQKIT ISDIINPTIT
Hmw2com    GLITVGKDGS VNLIGGKVKN EGVISVNGGS ISLLAGQKIT ISDIINPTIT

           251                                                 300
Hmw3com    YSIAAPENEA INLGDIFAKG GNINVRAATI RNKGKLSADS VSKDKSGNIV
```

FIG.10D.

```
Hmw4com   YSIAAPENEA INLGDIFAKG GNINVRAATI RNKGKLSADS VSKDKSGNIV
Hmw1com   YSIAAPENEA VNLGDIFAKG GNINVRAATI RNKGKLSADS VSKDKSGNIV
Hmw2com   YSIAAPENEA VNLGDIFAKG GNINVRAATI RNKGKLSADS VSKDKSGNIV

          301                                              350
Hmw3com   LSAKEGEAEI GGVISAQNQQ AKGGKLMITG DKVTLKTGAV IDLSGKEGGE
Hmw4com   LSAKEGEAEI GGVISAQNQQ AKGGKLMITG DKVTLKTGAV IDLSGKEGGE
Hmw1com   LSAKEGEAEI GGVISAQNQQ AKGGKLMITG DKVTLKTGAV IDLSGKEGGE
Hmw2com   LSAKEGEAEI GGVISAQNQQ AKGGKLMITG DKVTLKTGAV IDLSGKEGGE

          351                                              400
Hmw3com   TYLGGDERGE GKNGIQLAKK TTLEKGSTIN VSGKEKGGRA IVWGDIALID
Hmw4com   TYLGGDERGE GKNGIQLAKK TTLEKGSTIN VSGKEKGGRA IVWGDIALID
Hmw1com   TYLGGDERGE GKNGIQLAKK TTLEKGSTIN VSGKEKGGRA IVWGDIALID
Hmw2com   TYLGGDERGE GKNGIQLAKK TTLEKGSTIN VSGKEKGGRA IVWGDIALID
```

FIG.10E.

```
            401                                                   450
Hmw3com     GNINAQGK.D IAKTGGFVET SGHYLSIDDN AIVKTKEWLL DPENVTIEAP
Hmw4com     GNINAQGS.D IAKTGGFVET SGHDLSIGDD VIVDAKEWLL DPDDVSIETL
Hmw1com     GNINAQGSGD IAKTGGFVET SGHDLFIKDN AIVDAKEWLL DPDNVTINAE
Hmw2com     GNINAQGSGD IAKTGGFVET SGHYLSIESN AIVKTKEWLL DPDDVTIEAE

            451                                                   500
Hmw3com     SASRVELGAD RNSHSAEVIK VTLKKNNTSL TTLTNTTISN LLKSAHVVNI
Hmw4com     TSGRNNTGEN QGYTTGDGTK ESPKGNSISK PTLTNSTLEQ ILRRGSYVNI
Hmw1com     TAGRSNTSED DEYTGSGNSA STPKRNKE.K TTLTNTTLES ILKKGTFVNI
Hmw2com     DPLRNNTGIN DEFPTGTGEA SDPKKNSELK TTLTNTTISN YLKNAWTMNI

            501                                                   550
Hmw3com     TARRKLTVNS SISIERGSHL ILHSEGQGGQ GVQIDKDITS .E...GGNLT
Hmw4com     TANNRIYVNS SINLSNGS.L TLHTK...RD GVKINGDITS NE...NGNLT
Hmw1com     TANQRIYVNS SINL.SNGSL TLWSEGRSGG GVEINNDITT GDDTRGANLT
Hmw2com     TASRKLTVNS SINGSNGSHL ILHSKGQRGG GVQIDGDIT. ...SKGGNLT
```

RECTIFIED SHEET (RULE 91)

FIG.10F.

```
           551                                                  600
Hmw3com    IYSGGWVDVH KNITLGS.GF LNITTKEGDI AFEDKSGR.. ..NNLTITAQ
Hmw4com    IKAGSWVDVH KNITLGT.GF LNIVAGDS.V AFEREGDKAR NATDAQITAQ
Hmw1com    IYSGGWVDVH KNISLGAQGN INITAKQD.I AFEKGSNQV. ......ITGQ
Hmw2com    IYSGGWVDVH KNITLD.QGF LNITA.AS.V AFEGGNNKAR DANNLTITAQ

           601                                                  650
Hmw3com    GTITSG.NSN GFRFNNVSLN SLGGKLSFTD SREDRGRRTK GNISNKFDGT
Hmw4com    GTITVNKDDK QFRFNNVSIN GTGKGLKFIA NQN....... .NFTHKFDGE
Hmw1com    GTIT.SGNQK GFRFNNVSLN GTGSGLQFTT KRTN.....K YAITNKFEGT
Hmw2com    GTVTITGEGK DFRANNVSLN GTGKGLNIIS SVNN...... ..LTHNLSGT

           651                                                  700
Hmw3com    LNISGTVDIS MKAPKVSWFY RD.KGRTYWN VTTLNVTSGS KFNLSIDSTG
Hmw4com    INISGIVTIN QTTKKDVKYW NA.SKDSYWN VSSLTLNTVQ KFTF.IKFVD
Hmw1com    LNISGKVNIS MVLPKNESGY DKFKGRTYWN LTSLNVSESG EFNLTIDSRG
```

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FIG. 10G.

```
Hmw2com  INISGNITIN QTTRKNTSYW QTSHD.SHWN VSALNLETGA NFTF.IKYIS

         701                                              750
Hmw3com  SGSTG...PS IRNA..ELNG ITFN....KA TFNIAQGSTA NFSIKASIMP
Hmw4com  SGSNS...QD LRSSRRSFAG VHFNGIGGKT NFNIGANAKA LFKLKPNAAT
Hmw1com  SDSAGTLTQ. ....PYNLNG ISFN...KDT TFNVERNARV NFDIKAPIGI
Hmw2com  SNSKGLTTQY RSSAGVNFNG V..N...GNM SFNLKEGAKV NFKLKPNENM

         751                                              800
Hmw3com  FKSNANYAL. FNEDISVSG. .GGSVNFKLN ASSSNIQTPG VIIKSQNFNV
Hmw4com  DPKKELPIT. FNANITATGN SDSSVMFDIH A...NLTSRA AGINMDSINI
Hmw1com  NKYSSLNYAS FNGNISVSG. .GGSVDFTLL ASSSNVQTPG VVINSKYFNV
Hmw2com  NTSKPLPI.R FLANITATG. .GGSVFFDIY ANHS...GRG AELKMSEINI

         801                                              850
Hmw3com  SGGSTLNLKA EGSTETAFSI ENDLNLNATG GNITIRQVEG T..DSRVNKG
Hmw4com  TGGLDFSITS HNRNSNAFEI KKDLTINATG SNFSLKQTKD SFYNEYSKHA
```

FIG. 10H.

```
Hmw1com   STGSSLRFKT SGSTKTGFSI EKDLTLNATG GNITLLQVEG T..DGMIGKG
Hmw2com   SNGANFTLNS HVRGDDAFKI NKDLTINATN SNFSLRQTKD DFYDGYARNA

          851                                                900
Hmw3com   VAAKKNITFK GGNITFGSQK ATTEIKGNVT INKNTNATLR GANFAEN...
Hmw4com   INSSHNLTIL GGNVTLGGEN SSSSITGNIN ITNKANVTLQ ADTSNSNTGL
Hmw1com   IVAKKNITFE GGNITFGSRK AVTEIEGNVT INNNANVTLI GSDFDNHQ..
Hmw2com   INSTYNISIL GGNVTLGGQN SSSSITGNIT IEKAANVTLE ANNAPNQQNI

          901                                                950
Hmw3com   KSPLNIAGNV INNGNLTTAG SIINIAGNLT VSKGANLQAI TNYTFNVAGS
Hmw4com   KKRTLTLGNI SVEGNLSLTG ANANIVGNLS IAEDSTFKGE ASDNLNITGT
Hmw1com   KPLTIKKDVI INSGNLTAGG NIVNIAGNLT VESNANFKAI TNFTFNVGGL
Hmw2com   RDRVIKLGSL LVNGSLSLTG ENADIKGNLT ISESATFKGK TRDTLNITGN

          951                                               1000
```

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FIG.10I.

```
              1001                                                   1050
Hmw3com  FDNNGASNIS IARGGAKFK. DINNTSSLNI TTNSDTTYRT IIKGNISNKS
Hmw4com  FTNNGTANIN IKQGVVKLQG DINNKGGLNI TTNASGTQKT IINGNITNEK
Hmw1com  FDNKGNSNIS IAKGGARFK. DIDNSKNLSI TTNSSSTYRT IISGNITNKN
Hmw2com  FTNNGTAEIN ITQGVVKLG. NVTNDGDLNI TTHAKRNQRS IIGGDIINNK

         1001                                              1050
Hmw3com  GDLNIIDKKS DAEIQIGGNI SQKEGNLTIS SDKVNITNQI TIKAGVEGGR
Hmw4com  GDLNIKNIKA DAEIQIGGNI SQKEGNLTIS SDKVNITNQI TIKAGVEGGR
Hmw1com  GDLNITNEGS DTEMQIGGDI SQKEGNLTIS SDKINITKQI TIKAGVDGEN
Hmw2com  GSLNITDSNN DAEIQIGGNI SQKEGNLTIS SDKINITKQI TIKKGIDGED

         1051                                              1100
Hmw3com  SDSSEAENAN LTIQTKELKL AGDLNISGFN KAEITAKNGS DLTIGNASGG
Hmw4com  SDSSEAENAN LTIQTKELKL AGDLNISGFN KAEITAKNGS DLTIGNASGG
Hmw1com  SDSDATNNAN LTIKTKELKL TQDLNISGFN KAEITAKDGS DLTIGNTNSA
Hmw2com  SSSDATSNAN LTIKTKELKL TEDLSISGFN KAEITAKDGR DLTIGNSNDG
```

FIG. 10J.

```
          1101                                                    1150
Hmw3com   N..ADAKKVT FDKVKDSKIS TDGHNVTLNS EVKT..SNGS SNAGNDNSTG
Hmw4com   N..ADAKKVT FDKVKDSKIS TDGHNVTLNS EVKT..SNGS SNAGNDNSTG
Hmw1com   D.GTNAKKVT FNQVKDSKIS ADGHKVTLIIS KVETSGSNNN TEDSSDNNAG
Hmw2com   NSGAEAKKVT FNNVKDSKIS ADGHNVTLNS KVKTSSSNGG RESNSDNDTG

          1151                                                    1200
Hmw3com   LTISAKDVTV NNNVTSHKTI NISAAAGNVT TKEGTTINAT TGSVEVTAQN
Hmw4com   LTISAKDVTV NNNVTSHKTI NISAAAGNVT TKEGTTINAT TGSVEVTAQN
Hmw1com   LTIDAKNVTV NNNITSHKAV SISATSGEIT TKTGTTINAT TGNVEIT...
Hmw2com   LTITAKNVEV NKDVTSLKTV NITA.SEKVT TTAGSTINAT NGKASIT...

          1201                                                    1250
Hmw3com   GTIKGNITSQ NVTVTATENL VTTENAVINA TSGTVNISTK TGDIKGGIES
Hmw4com   GTIKGNITSQ NVTVTATENL VTTENAVINA TSGTVNISTK TGDIKGGIES
Hmw1com   .......... .......... .......... ........AQ TGDIKGGIES
```

FIG. 10K.

```
Hmw2com   .......... .......... .......... ........TK T.........

          1251                                              1300
Hmw3com   TSGNVNITAS GNTLKVSNIT GQDVTVTADA GALTTTAGST ISATTGNANI
Hmw4com   TSGNVNITAS GNTLKVSNIT GQDVTVTADA GALTTTAGST ISATTGNANI
Hmw1com   SSGSVTLTAT EGALAVSNIS GNTVTVTANS GALTTLAGST IKG.TESVTT
Hmw2com   .......... .......... .......... .......... ..........

          1301                                              1350
Hmw3com   TTKTGDINGK VESSSGSVTL VATGATLAVG NISGNTVTIT ADSGKLTSTV
Hmw4com   TTKTGDINGK VESSSGSVTL VATGATLAVG NISGNTVTIT ADSGKLTSTV
Hmw1com   SSQSGDIG.. .......... .........G TISGGTVEVK ATESLTTQSN
Hmw2com   ....GDIS.. .......... .........G TISGNTVSVS ATVDLTTKSG

          1351                                              1400
Hmw3com   GSTINGTNSV TTSSQSGDIE GTISGNTVNV TASTGDLTIG NSAKVEAKNG
Hmw4com   GSTINGTNSV TTSSQSGDIE GTISGNTVNV TASTGDLTIG NSAKVEAKNG
```

FIG. 10L.

```
Hmw1com   SKIKATTGEA NVTSATGTIG GTISGNTVNV TANAGDLTVG NGAEINATEG
Hmw2com   SKIEAKSGEA NVTSATGTIG GTISGNTVNV TANAGDLTVG NGAEINATEG

          1401                                              1450
Hmw3com   AATLTAESGK LTTQTGSSIT SSNGQTTLTA KDSSIAGNIN AANVTLNTTG
Hmw4com   AATLTAESGK LTTQTGSSIT SSNGQTTLTA KDSSIAGNIN AANVTLNTTG
Hmw1com   AATLTTSSGK LTTEASSHIT SAKGQVNLSA QDSSVAGSIN AANVTLNTTG
Hmw2com   AATLTATGNT LTTEAGSSIT STKGQVDLLA QNSSIAGNIN AANVTLNTTG

          1451                                              1500
Hmw3com   TLTTTGDSKI NATSGTLTIN AKDAKLDGAA SGDRTVVNAT NASGSGNVTA
Hmw4com   TLTTTGDSKI NATSGTLTIN AKDAKLDGAA SGDRTVVNAT NASGSGNVTA
Hmw1com   TLTTVKGSNI NATSGTLTIN AKDAELNGAA LGNHTVVNAT NANGSGSVIA
Hmw2com   TLTTVAGSDI KATSGTLTIN AKDAKLNGDA SGDSTEVNAV NASGSGSVTA

          1501                                              1550
```

RECTIFIED SHEET (RULE 91)

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FIG. 10L.

```
Hmw3com   KTSSSVNITG DLNTINGLNI ISENGRNTVR LRGKEIDVKY IQPGVASVEE
Hmw4com   KTSSSVNITG DLNTINGLNI ISENGRNTVR LRGKEIDVKY IQPGVASVEE
Hmw1com   TTSSRVNITG DLITINGLNI ISKNGINTVL LKGVKIDVKY IQPGIASVDE
Hmw2com   ATSSSVNITG DLNTVNGLNI ISKDGRNTVR LRGKEIEVKY IQPGVASVEE

          1551                                              1600
Hmw3com   VIEAKRVLEK VKDLSDEERE TLAKLGVSAV RFVEPNNAIT VNTQNEFTTK
Hmw4com   VIEAKRVLEK VKDLSDEERE TLAKLGVSAV RFVEPNNAIT VNTQNEFTTK
Hmw1com   VIEAKRILEK VKDLSDEERE ALAKLGVSAV RFIEPNNTIT VDTQNEFATR
Hmw2com   VIEAKRVLEK VKDLSDEERE TLAKLGVSAV RFVEPNNTIT VNTQNEFTTR

          1601                           1632
Hmw3com   PSSQVTISEG KACFSSGNGA RVCTNVADDG QQ        (SEQ ID NO: 9)
Hmw4com   PSSQVTISEG KACFSSGNGA RVCTNVADDG QQ        (SEQ ID NO: 10)
Hmw1com   PLSRIVISEG RACFSNSDGA TVCVNIADNG R.        (SEQ ID NO: 2)
Hmw2com   PSSQVIISEG KACFSSGNGA RVCTNVADDG QP        (SEQ ID NO: 4)
```

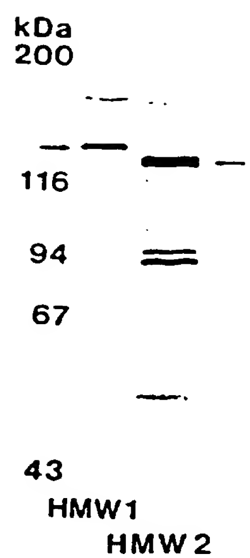

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FIG. ~~2~~. Western immunoblot assay of phage lysates containing either the HMW1 or HMW2 recombinant proteins. Lysates were probed with an *E. coli*-absorbed adult serum sample with high-titer antibody against high-molecular-weight proteins. The arrows indicate the major immunoreactive protein bands of 125 and 120 kDa in the HMW1 and HMW2 lysates, respectively.

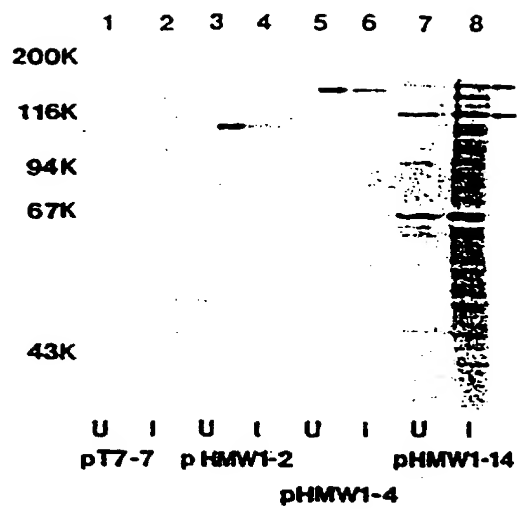

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FIG. 3. Western immunoblot assay of cell sonicates prepared from *E. coli* transformed with plasmid pT7-7 (lanes 1 and 2), pHMW1-2 (lanes 3 and 4), pHMW1-4 (lanes 5 and 6), or pHMW1-14 (lanes 7 and 8). The sonicates were probed with an *E. coli*-absorbed adult serum sample with high-titer antibody against high-molecular-weight proteins. Lanes labeled U and I represent sonicates prepared before and after induction of the growing samples with IPTG, respectively. The arrows indicate protein bands of interest as described in the text.

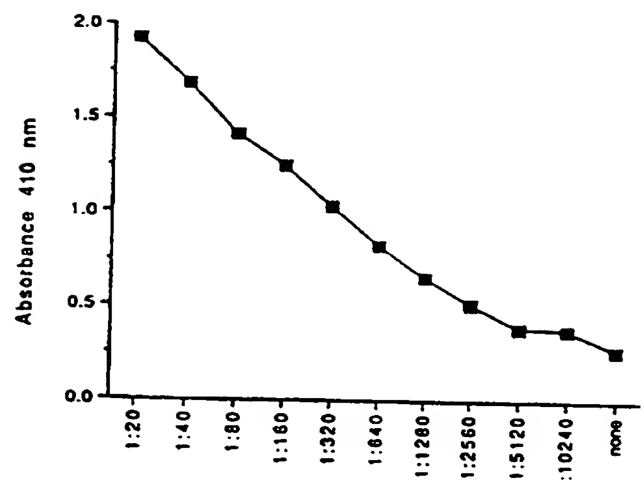

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FIG. ~~6~~. ELISA with rHMW1 antiserum assayed against purified filamentous hemagglutinin of *B. pertussis*. Ab, antibody.

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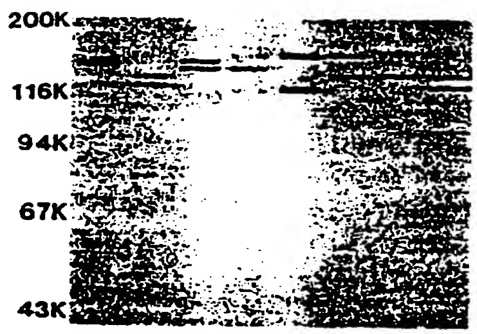

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FIG. 17. Western immunoblot assay of cell sonicates from a panel of epidemiologically unrelated nontypeable *H. influenzae* strains. The sonicates were probed with rabbit antiserum prepared against HMW1-4 recombinant protein. The strain designations are indicated by the numbers below each lane.

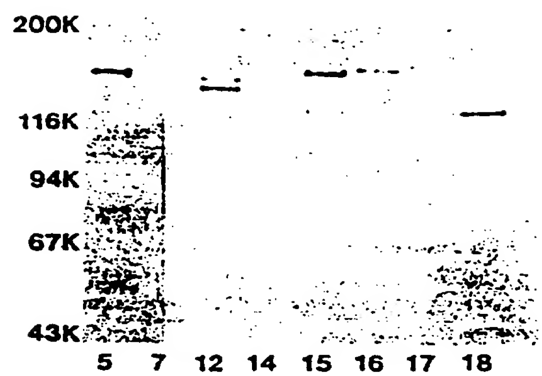

FIG. 15. Western immunoblot assay of cell sonicates from a panel of epidemiologically unrelated nontypeable *H. influenzae* strains. The sonicates were probed with monoclonal antibody X3C, a murine IgG antibody which recognizes the filamentous hemagglutinin of *B. pertussis* (13). The strain designations are indicated by the numbers below each lane.

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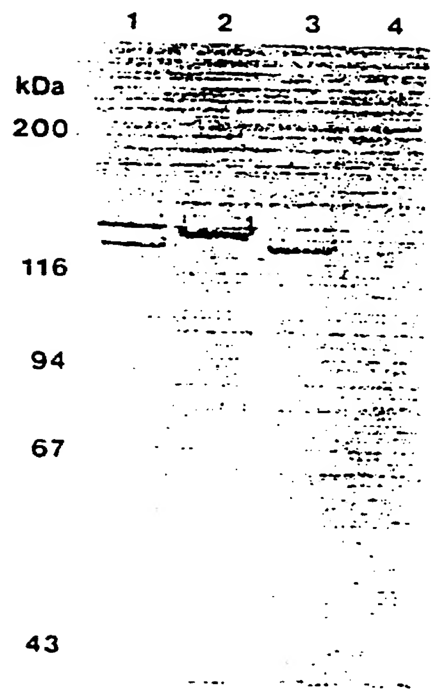

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FIG. 1. Immunoblot assay of cell sonicates of nontypable *H. influenzae* strain 12 derivatives. The sonicates were probed with rabbit antiserum prepared against HMW-1 recombinant protein. Lanes: 1, wild-type strain; 2, HMW-2⁻ mutant; 3, HMW-1⁻ mutant; 4, HMW-1⁻/HMW-2⁻ double mutant.

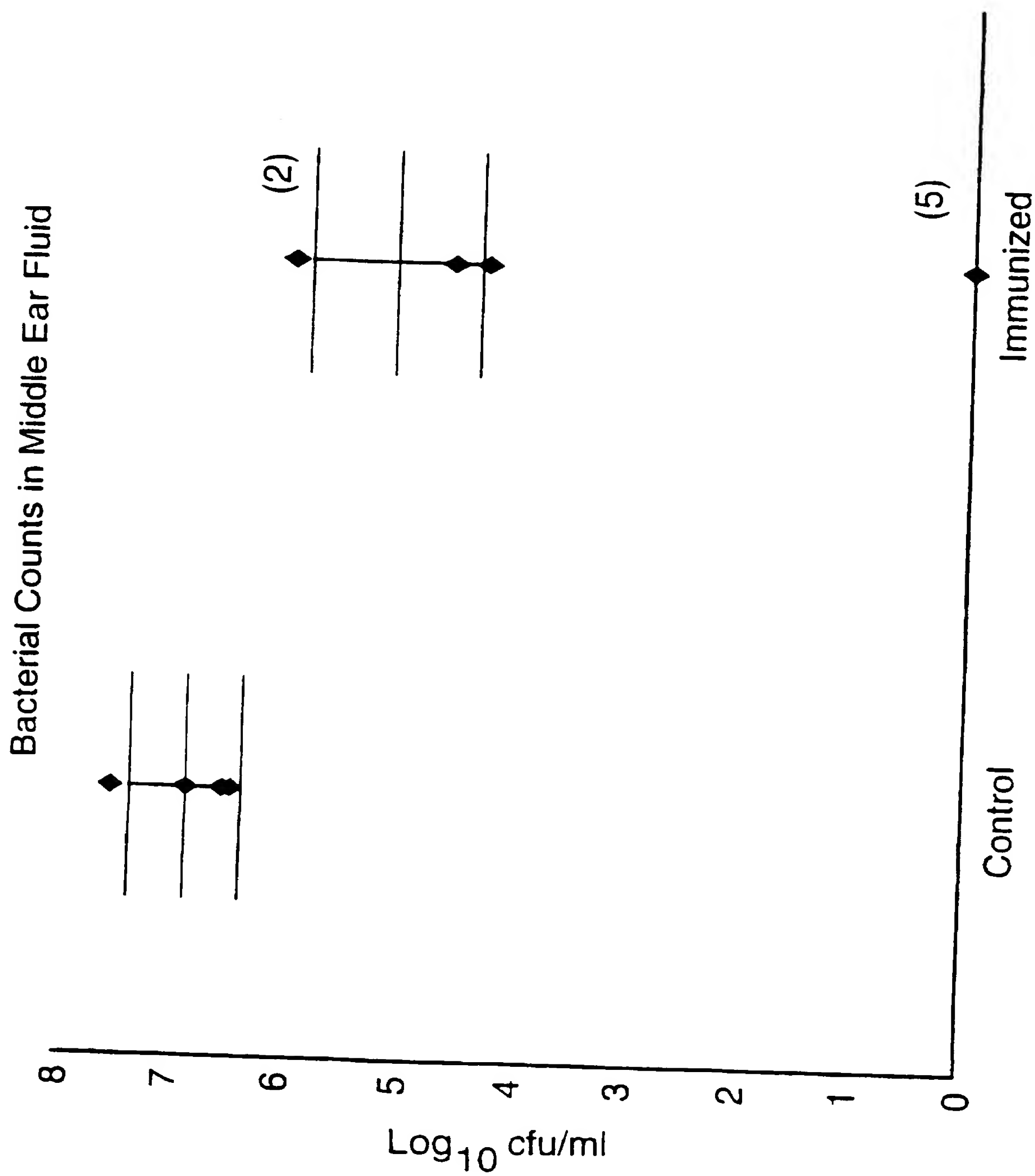
Bacterial Counts in Middle Ear Fluid
Log10 cfu/ml
8
7
6
5
4
3
2
1
0
(2)
(5)
Control
Immunized

FIG.17

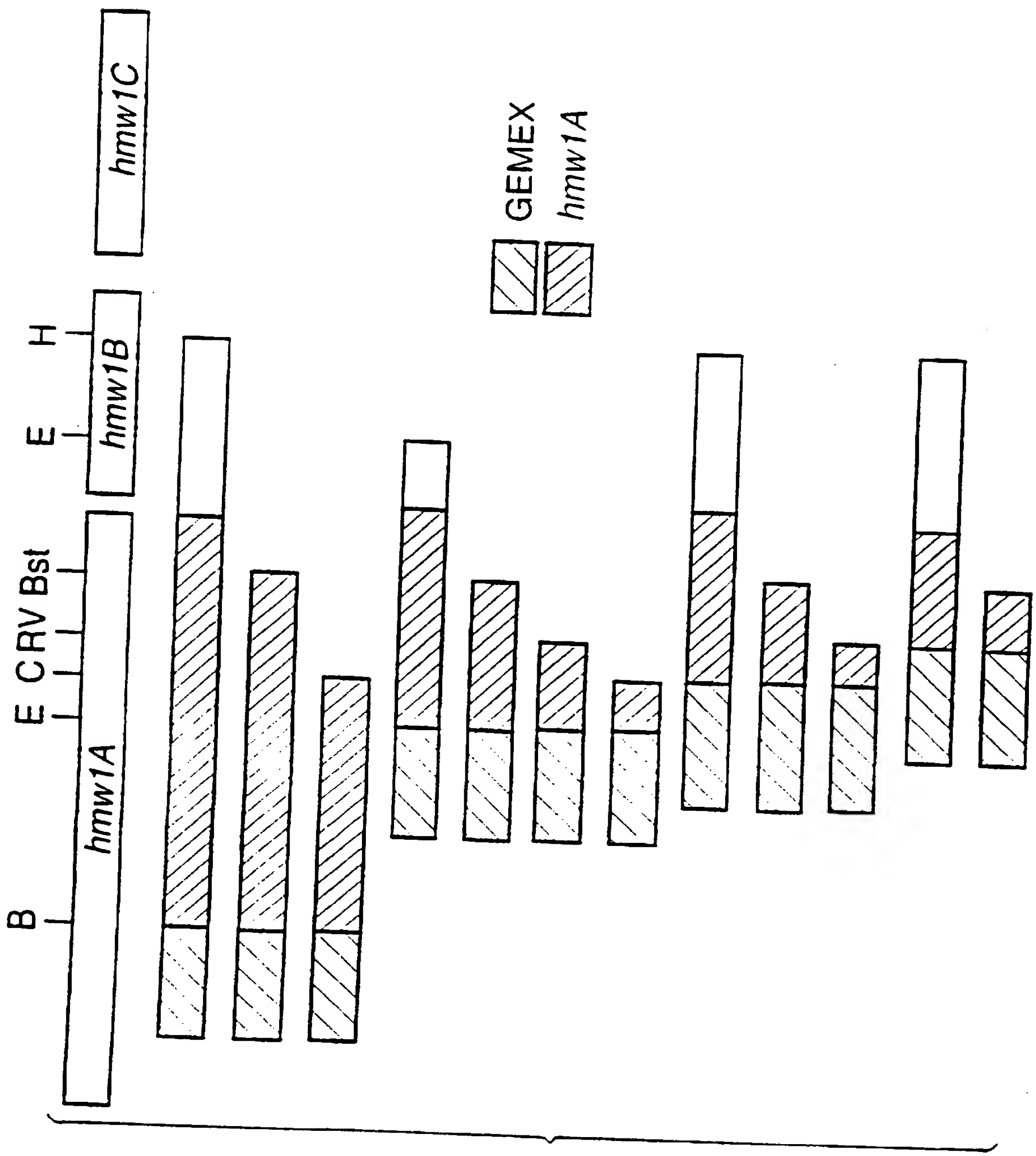

FIG.18

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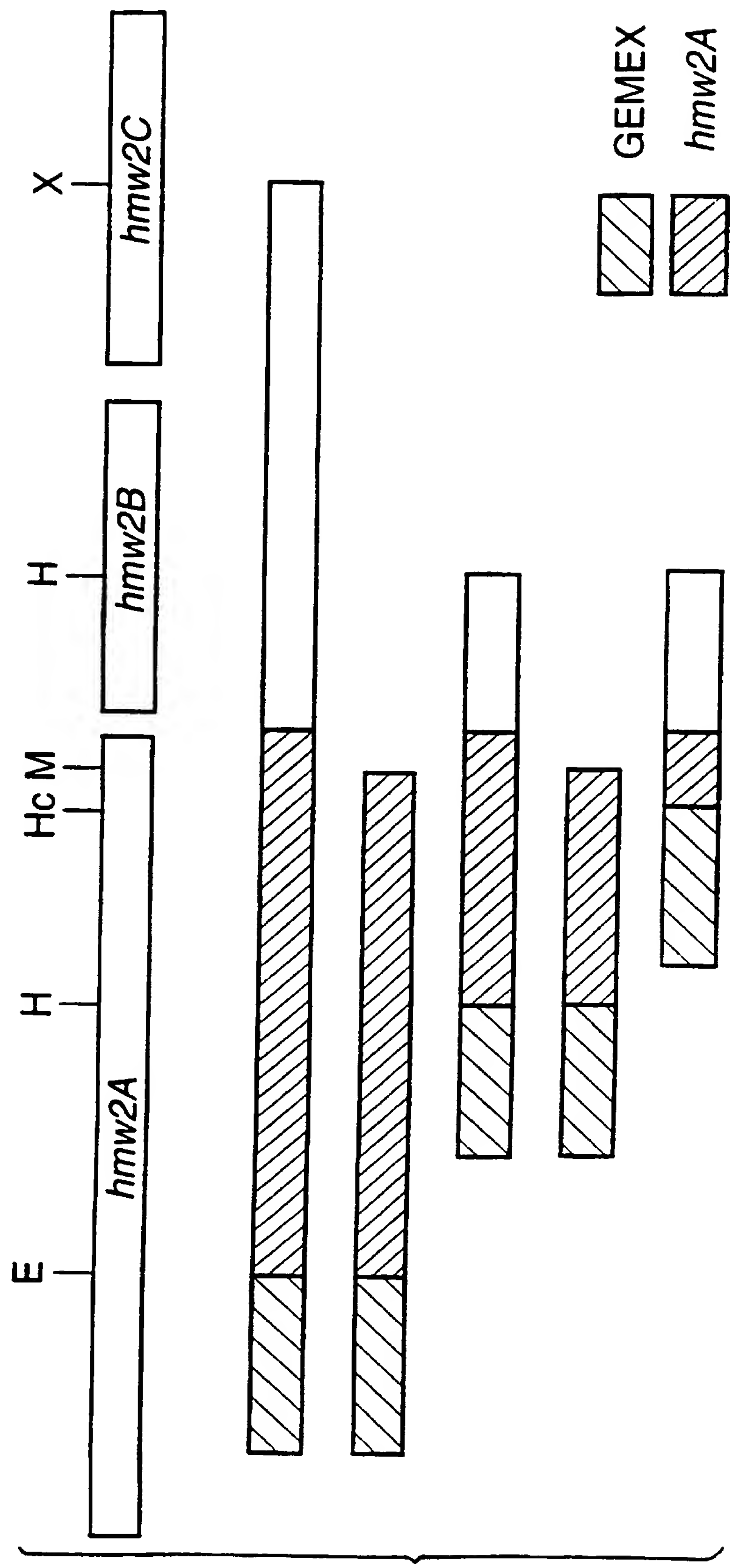

FIG.19

Immunoelectron microscopy with Mab AD6

Figure 20

Western immunoblot assay with Mab AD6 and HMW1A or HMW2A recombinant proteins

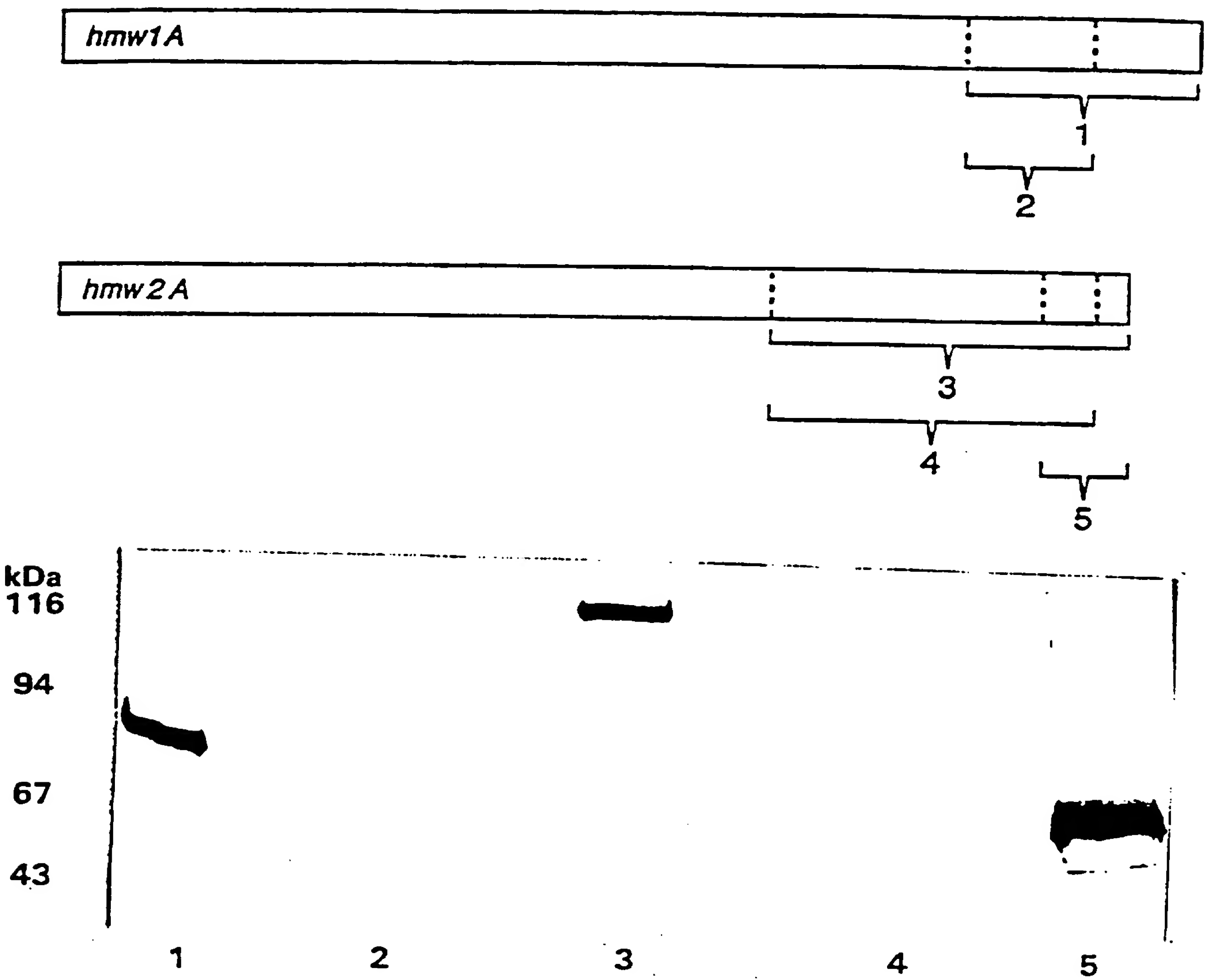

Figure 4 21

Western immunoblot assay with Mab 10C5 and HMW1A or HMW2A recombinant proteins

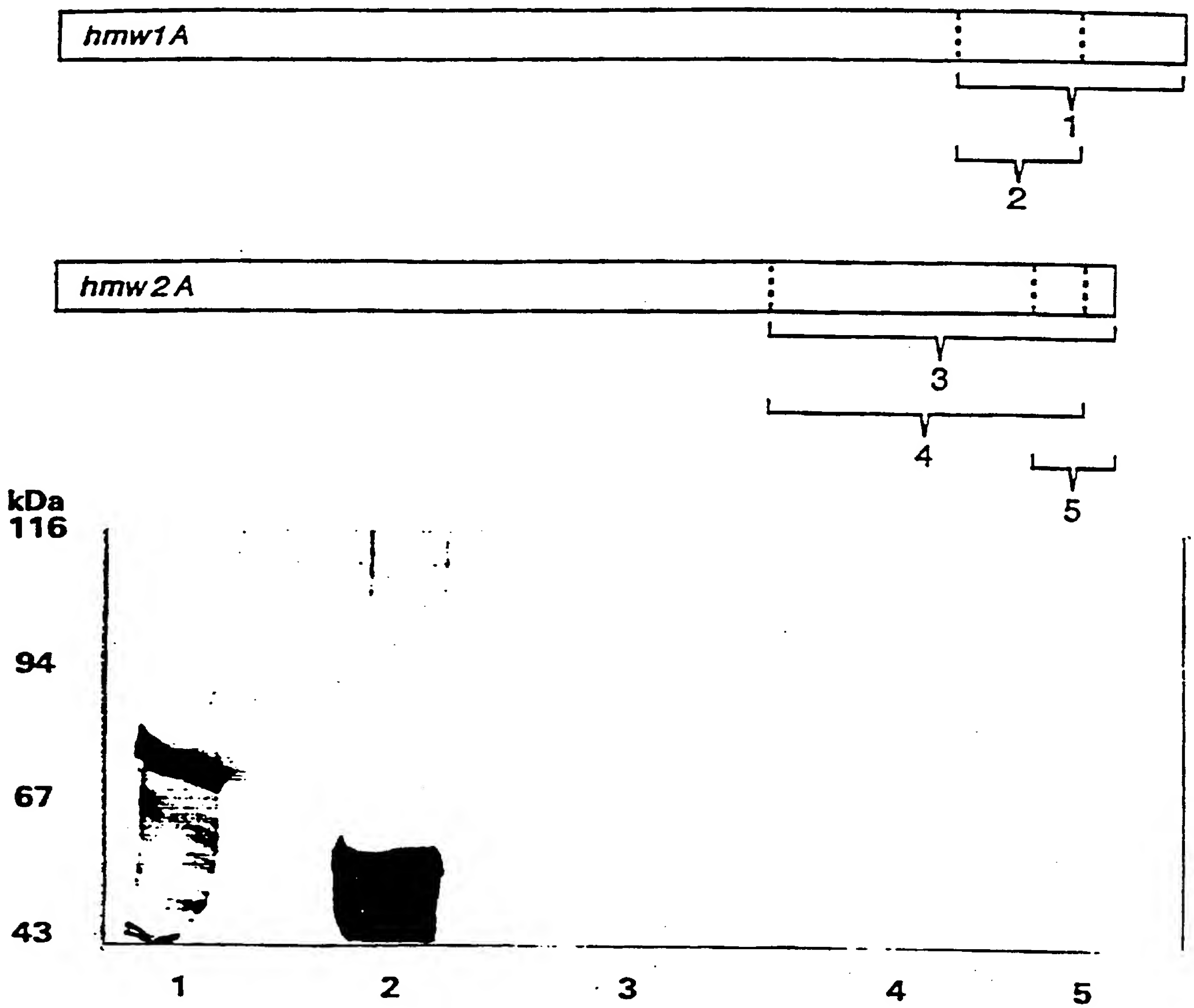

Fig 22

Western immunoblot assay with Mab AD6 and ten unrelated nontypable *Haemophilus influenzae*

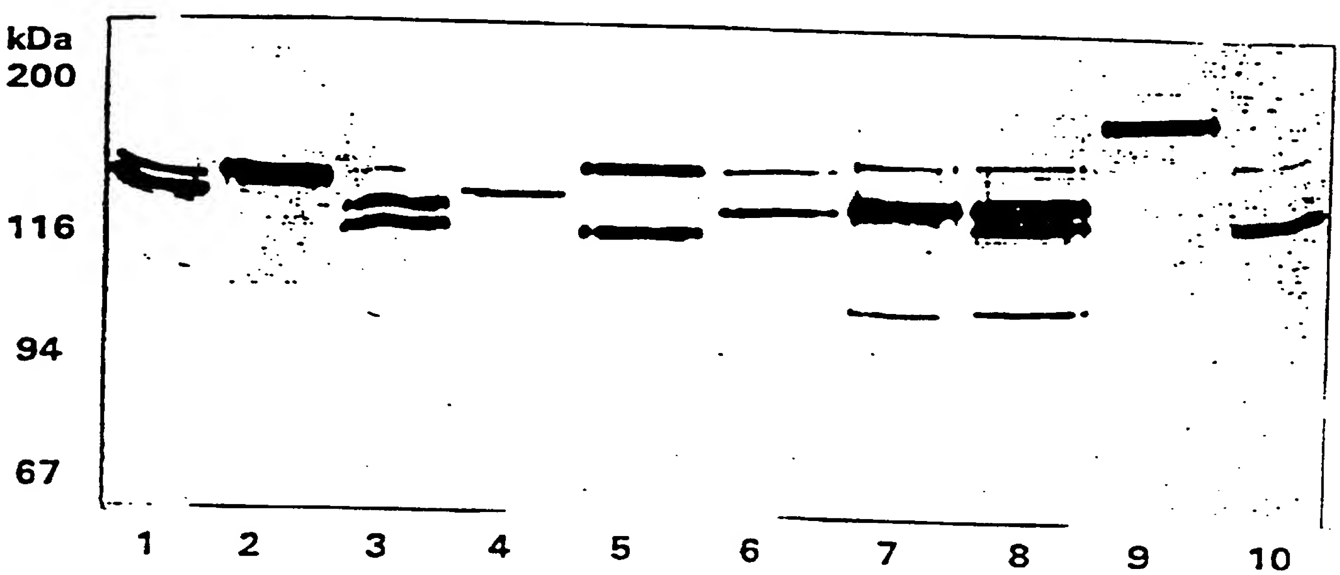

Figure 5 23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/04707

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/02, 21/04; C12P 21/06; A61K 39/102
US CL :536/23.1, 23.4, 23.7, 24.3, 24.33; 435/69.1; 424/256.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 23.4, 23.7, 24.3, 24.33; 435/69.1; 424/256.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, CAS, MEDLINE, BIOSIS, MPSRCH
search terms: haemophilus influenzae, h. influenzae, high molecular weight, hmw

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93/19090 A1 (BARENKAMP) 30 September 1993, entire document.	1-4
X --- Y	BARENKAMP et al. Cloning, Expression, and DNA Sequence Analysis of Genes Encoding Nontypeable *Haemophilus influenzae* High-Molecular-Weight Surface-Exposed Proteins Related to Filamentous Hemagglutinin of *Bordetella pertussis*. Infection and Immunity. April 1992, Volume 60, No. 4, pages 1302-1313, entire document.	2-4 ------ 1
X	WO 94/21290 A1 (BARENKAMP) 29 September 1994, entire document.	1-4

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 14 MAY 1997	Date of mailing of the international search report 10 JUN 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JENNIFER SHAVER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US97/04707

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	BARENKAMP et al. Genes Encoding High-Molecular-Weight Adhesion Proteins of Nontypeable *Haemophilus influenzae* Are Part of Gene Clusters. Infection and Immunity. August 1994, Volume 62, No. 8, pages 3320-3328, entire document.	1 ---- 2-4

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.
Group I, claim(s) 1-4, drawn to DNA and vectors.
Group II, claim(s) 5-9, 12 and 13, drawn to proteins.
Group III, claim(s) 10 and 11, drawn to conjugate molecules.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of Group I is DNA encoding a high molecular weight protein of *Haemophilus influenzae*. This DNA is separate and independent from the proteins of Group II and the conjugates of Group III as it is biologically, chemically and structurally different. The special technical feature of Group II is high molecular weight proteins of *Haemophilus influenzae* which are separate and independent from Group III as they are not linked to an antigen, hapten or polysaccharide. These peptides have different immunological properties then the conjugates of Group III. The conjugates of Group III are different structurally from the proteins of Group II and may be used as multivalent vaccines. The DNA of Group I may be used for purposes other than encoding the proteins of Group II, i.e., as probes or primers in detection methods. For these reasons, the inventions of Groups I-III are shown to have different properties with no common link between them.